A study of controlled drug delivery using low constant current electrophoresis

D'Emanuele, Antony John

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A STUDY OF

CONTROLLED DRUG DELIVERY USING

LOW CONSTANT CURRENT ELECTROPHORESIS

submitted by

Antony John D'Emanuele, BPharm, MRPharmS.

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This research has been carried out in the School of Pharmacy and Pharmacology of the University of Bath under the supervision of J. N. Staniforth, BSc, PhD, MRPharmS.

ProQuest LLC
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"If you do not expect the unexpected you will not find it; for it is hard to be sought out, and difficult".

Heraclitus ca 550-475 BC

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TO MY MOTHER AND FATHER

FOR YOUR LOVE AND UNFAILING SUPPORT

SUMMARY

The principal aim of this study was to examine the feasibility and potential of an electrophoretically modulated constant current drug delivery device.

In addition to discussing the rationale for controlled drug delivery, the introduction reviews recent advances that have been made in this area. The skin is considered as a potential route for drug delivery, together with the properties and uses of poly(2-hydroxyethyl methacrylate) (PHEMA) as a material for use in drug delivery devices. The experimental work is divided into three sections, dealing with the characterization of materials and especially the properties of PHEMA polymer films; investigation of the use of low constant currents to control the transport of a model drug (propranolol HCl (PHC)) through films of PHEMA; and lastly an investigation of a model transdermal electrophoretic device.

PHEMA was prepared by chemically initiated polymerization and some of its physico-chemical properties examined. The diffusion of PHC through films of crosslinked PHEMA was investigated and found to be anomalous with respect to previously reported behaviour. The adsorption isotherm of PHC on PHEMA in aqueous solution indicated an interaction between PHC and PHEMA. The relationship between the anomalous diffusion behaviour of PHC through PHEMA films and the interaction between PHC and PHEMA was examined, in particular the involvement of negatively charged sites in PHEMA. It was found that charged ionic sites in PHEMA could only partly explain the anomalous

diffusion behaviour of PHC and the interaction in solution between PHC and PHEMA.

The use of low constant currents (0 to 2.4 mA) to control the transport of PHC across films of PHEMA was examined. An automated model system was developed where flexible control over electrophoresis and data collection was demonstrated. It was found that the transport of PHC through PHEMA films could be modulated predictably and reproducibly in a positive or negative manner. The degradation of PHC during electrophoresis was investigated. Several factors affecting the electrophoretic transport of PHC such as PHEMA crosslinker content, ionic strength and temperature were also investigated. Application of an electrophoretic current during the diffusion lag time was found to produce a different effect to that found when electrophoresis was carried out during steady state diffusion. It was demonstrated that feedback control could be used to effect the release of PHC using a computer-controlled model system.

The permeability coefficient of PHC through hairless mouse skin was determined and the effect of two permeation enhancers (sodium dodecyl sulphate and Azone) on the transport of PHC through hairless mouse skin examined. The information derived from these experiments was used to investigate an in vitro model of a transdermal electrophoretic device. The model used indicated that controlled drug delivery though the skin may be limited due to the long lag times involved.

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Chapter 1

Introduction and Theory

1.1 Evolution of Drug Delivery Devices

Drug delivery devices may be broadly subdivided into three categories, firstly the conventional single dose or bolus medicine, secondly the constant release type of device which contain the equivalent of several single doses and where the drug delivery rate is a simple function of time (eg. t^{o}, $t^{-1/2}$ or t^{1}), and finally the controlled drug delivery device where the rate may be modulated to provide an ever-changing drug delivery profile. The historical development of these groups of devices reflects the awareness that has developed regarding the therapeutic importance of dosage forms, the optimum therapeutic effect of many drugs is dependent on the form and delivery rate as much as on the intrinsic pharmacological activity of the drug [1]. It has become clear that the use of conventional dosage forms has resulted in many drugs not realizing their full therapeutic potential [1]. In addition to development of new dosage forms occurring exclusively as a result of therapeutic requirements, it is also the case that industry has invested heavily in a search for new dosage forms due to the enormous expense involved in developing new drug entities [2,3,4].

The route by which delivery devices administer drug to the body has also been an area of intense research interest [1,5,6]. The majority of present dosage forms are administered via the oral route; however other routes of administration are also used and these include ocular, rectal, nasal, pulmonary and transdermal applications [1]. Of these routes, a disproportionate research interest has been shown recently in the transdermal route, due to the significant potential advantages of transdermal delivery (section 1.5).

Many controlled drug delivery devices rely on polymers as a major component [1,7,8]. New polymeric materials are continuously being developed to add to the extensive range of polymers which researchers in the field already have at their disposal [9,10,11]. The unique physico-chemical or biochemical properties that individual polymeric materials offer have been exploited in the development of many drug delivery devices. A polymer that has been examined as a component in a number of drug delivery devices is poly(2-hydroxyethyl methacrylate) (PHEMA), this polymer will be discussed below in section 1.6.

1.2 Rationale for Controlled Drug Delivery

The most common types of drug delivery device currently used are based on the conventional repeated single dose or bolus type of delivery, the oral route of delivery being the primary route of administration of these devices. The most popular of these dosage forms are tablets, accounting for approximately 70% of all ethical pharmaceutical preparations produced [12]. However, included amongst the many other conventional dosage forms are solutions, suspensions, creams, ointments, inhalation aerosols, suppositories, pessaries and bolus injections. These conventional dosage forms can suffer from a number of therapeutic disadvantages [1,5,6,13-16]. A disadvantage which is also shared by most types of conventional medicines or newly developed drug delivery devices is that drug delivered by such dosage forms often acts systemically as well as on a specific target. Although the disadvantages associated with the use of non-targeted drug delivery are often not therapeutically significant and at worst result in the occurrence of only minor side effects, with certain

drugs such as those with cytotoxic action, unwanted side effects are a major problem with non-targeted delivery [17]. A number of approaches have been attempted as a means of targeting drugs to specific sites in order to avoid systemic side effects, some of these methods include the use of monoclonal antibodies [18,19], soluble polymers [20], and liposomes [21]. If research into drug targeting is successful, there will be an obvious need for suitable drug delivery devices. A major problem that is also shared by both conventional delivery devices and newly researched delivery devices concerns administration via the oral route. Orally administered drugs, once absorbed, pass through the hepatic system where they are susceptible to metabolism; this phenomenon is known as the first-pass effect. First-pass metabolism, as well as being potentially extensive, can result in a great variation in therapeutic drug concentrations between individuals and it is this major problem which has prompted workers to investigate other routes of administration, one of which, the transdermal route (section 1.5), has received widespread attention in recent years. A major problem with conventional delivery devices that has been recognised regards patient compliance with dosage regimes; an example of this concerns digoxin therapy where significant differences were found in plasma digoxin levels between compliant and non-compliant patients [22]. Examples can be found amongst drugs in common use which possess considerable therapeutic potential, yet frequently remain unused or underutilized when therapy would be too complex or burdensome for the patient [1].

Probably the main disadvantage associated with the use of existing medicines regards the plasma drug concentration profile produced by many of these conventional dosage forms. This problem has

become a significant driving force for research focussing on new drug delivery methods. When a single oral dose is administered, an initial burst of plasma drug levels occurs which may exceed the toxic level of the drug, the plasma level of drug then decays to a value below the minimum effective concentration [1,13]. It is apparent that the plasma drug levels may therefore be within the optimum therapeutic levels, the so called therapeutic window, for a relatively short period of time compared to the total time of residence of drug within the body. In order to maintain therapeutic levels of drug, repeated administration of the dosage form is required. Depending on the pharmacokinetics of a drug, particularly its biological half life, repeated administration of a single dose can result in a characteristic see-saw variation of plasma drug levels. The plasma drug levels oscillate within the therapeutic window, but allowing periods when a toxic level may be maintained, and also periods where the concentration may be below the minimum effective concentration [13]. This has precluded the clinical use of many promising drugs with very short biological half-lives or very narrow therapeutic indices [23]. Constant release delivery devices were developed primarily to avoid this type of see-saw effect and thus maintain constant plasma levels within the therapeutic window [1]. A review by Urquhart [23] considers the therapeutic consequences of having the see-saw type of variation in plasma drug levels found with most conventional delivery devices. It should be emphasized, however, that theoretically constant drug plasma levels may be obtained with conventional bolus dosage forms if the dose quantity and frequency were adjusted appropriately, but this would require dosing to be so frequent for most drugs that such an approach would be impractical

and disadvantageous [23]. Conventional drug delivery forms are often sensitive to environmental factors [6], for example, oral dosage forms, where the drug delivery rate may be affected by such factors as gastric motility and pH [6,24]. Constant release devices are designed to maintain constant plasma drug levels for periods of days, months or even years [25], and also to overcome some of the disadvantages associated with conventional delivery devices, which have already been discussed. An example where decreased side effects may be produced by maintaining a steady plasma level is the formulation of hyoscine hydrobromide as a transdermal delivery system known as the Scopoderm TTS device [1]. The side effects produced by conventional medicines containing hyoscine HBr such as dryness of the mouth, dizziness, tachycardia and drowsiness are found at a lower level of incidence with the transdermal device, although the TTS is equally effective in the treatment of motion sickness [1,26]. The use of constant release devices could also lead to less wastage of drug as a result of more efficient drug delivery, which may be of particular importance with expensive drugs. However, it should be remembered that many novel constant delivery devices are expensive to fabricate which may be a limiting factor in their extensive therapeutic use. There are a number of potential problems however with constant release drug delivery devices. Many devices are based on polymeric materials, which may present problems due to low biocompatibility, particularly with regard to their use in implant devices [27,28,29]. These devices will inevitably have a reservoir holding a potentially fatal dose of drug if accidentally released all at once. Prevention of such "dose dumping" from these devices must be guaranteed, as must be the reproducibility and predictability of drug

release [30]. Another problem, which applies particularly to implants, is that it will probably not be possible to reduce or terminate drug delivery immediately if so required.

Constant delivery devices offer considerable advantages over conventional drug delivery devices as has been described; however, for certain drugs, constant delivery rates may still not be the optimum manner of delivery. The advent of therapeutically potent peptides, polypeptides and proteins [31] suggest that these potential new drugs are likely to require delivery to the body in a precisely controlled manner which may be optimum when delivery mirrors physiological release profiles [31,32,33]. In addition peptides possess several properties which require special attention in the development of delivery devices [34]. An example where controlled drug delivery may be desirable is with insulin therapy: normal human pancreatic beta-cells modulate the secretion of insulin into the hepatic portal vein, dependent on the bodies demands on glucose, in order to maintain normoglycaemia. Conventional subcutaneous therapy used in the treatment of diabetes cannot maintain such control and stability [35]. Many of the long term complications that diabetics are prone to such as cardiovascular problems and neuropathies have been associated with poor control over glucose levels that conventional therapy provides, and evidence suggests that these complications may be delayed or decreased by insulin delivery systems which normalise glucose levels over a narrower range [36-41]. In order to improve therapy for diabetics, true controlled drug delivery devices are required where insulin output can be modulated. There are already a number of infusion pumps used for insulin therapy (section 1.3.6), and other suitable devices

are being investigated [42,43,44]. The ultimate goal must be for an artificial pancreas, where the output of a controlled delivery device is in response to feedback signals provided by a reliable glucose sensor.

The therapeutic benefits resulting from the use of true controlled delivery devices is also becoming evident from the results of current research which seems to indicate that the biological response to a given drug may vary according to the bodies circadian rhythms; a review by Pauly [45] presents the basis for research in this area of research, known as chronopharmacology. Chronopharmacologists have reported data which indicates that the absorption, metabolism and effects of various medicaments are likely to vary predictably with time, and it is being questioned whether constant plasma drug levels are necessarily the ideal for many drugs [45-48]. Controlled drug delivery devices able to respond to the bodies chronergy could result in drugs being used more effectively.

Propranolol HCl is a drug that may be a suitable candidate for a controlled drug delivery device. Conventional oral dosage forms of propranolol HCl are not entirely satisfactory due to low bioavailability and variability in plasma levels within individuals due to extensive first-pass metabolism [49,50,51]. Ideally a delivery route avoiding the hepatic circulation would thus be preferable. Controlled drug delivery of propranolol HCl may also be desirable as it has been reported that chronopharmacokinetic variations for propranolol HCl occur in man [52,53], as well as circadian rhythmicity in cardiovascular functions [46].

In 1971, the president of the Alza Corporation predicted that controlled drug delivery systems acting as artificial glands with

their own sense detecting feedback control would be developed in about 10 years [54]. Whilst his prediction has not yet been fully realized due to technology restraints, advances have been made since the statement was made in 1971 and it can certainly be envisaged that feedback controlled delivery devices could be used clinically within the next decade.

1.3 Constant and Controlled Drug Delivery Devices

A number of different techniques have been examined as potential methods for producing constant and controlled drug delivery devices. In this section a comparison of some of the various systems that are being currently researched or that have been successfully utilised to provide constant and controlled drug delivery are examined; however, most of the systems which will be discussed are still largely experimental.

1.3.1 Polymer Reservoir Systems

Polymer reservoir devices were one of the first types of constant delivery devices to be developed, they are usually composed of a drug reservoir encapsulated by a rate controlling polymeric membrane of well defined thickness [1,5,15,25,55,56]. The release of drugs from reservoir devices is governed by Fick's first law of diffusion, the rate of release being theoretically zero-order given that the activity of drug in the reservoir remains constant. However, once the drug reservoir starts to deplete, then the delivery rate decreases in a manner similar to first-order release [55]. The factors affecting the release rate from this type of device include the chemical and physical nature of the polymer membrane selected and

its thickness, the surface area of the device, and the solubility of the drug in the polymer membrane, assuming the diffusion coefficient of the drug is constant and that there are no stationary layers on the surface of the device [5,25,57]. There may be a 'burst' of drug release when these types of devices are used initially due to the rate controlling membrane being saturated with drug, however the delivery rate does subsequently stabilize and the release rate eventually becomes zero-order. A number of reservoir devices have been used clinically and these notably include the Progestasert device for the localized constant delivery of progesterone, the Transiderm Nitro patch for glyceryl trinitrate release, and the first clinically developed device, Ocusert [1,5,15,58]. The advantage of reservoir devices are that zero-order release kinetics are possible, however the disadvantages include the fact that these systems are generally not biodegradable and also leaks in the polymer membrane are potentially dangerous since "dose dumping" may occur [25]. A previously cited disadvantage of reservoir devices is that they are not suitable for the delivery of large molecules such as peptides [15,34], however, it has been reported that hydrogel copolymer membranes can be used as rate limiting membranes for the release of peptides [59].

1.3.2 Polymer Matrix Systems

Polymer matrix systems (monoliths) are constant delivery type devices, composed of a drug dispersed within an insoluble polymer matrix [5,15,25,30,60,61]. As with reservoir devices (section 1.3.1) the release of drugs is diffusion controlled and governed by Fick's first law of diffusion, however, whereas in the reservoir device the

concentration across a unit thickness of membrane may be constant, with a matrix system the concentration gradient is time dependent. This can be seen if the flat surface on a rectangular slab of a monolith device is considered, in which the drug is immobilised. If the drug is considered to be eluted a layer at a time (the drug must first dissolve before diffusing out), then the outermost layer of the device elutes first, then once this layer becomes exhausted, drug in the next layer starts to be depleted, thus the path length of diffusion increases with time and the concentration gradient decreases progressively [62,63]. For this simple type of device the result is a time dependent release rate, which is linearly related to $time^{-1/2}$ [62,64]. The actual equation describing release from matrix devices will depend on a number of factors [30,61], including the type of matrix device, the geometry and the initial drug loading. Examples of the types of matrix devices include dissolved matrix devices where the drug is dissolved in the matrix at a concentration below its saturation solubility, there is also the dispersed matrix device where the drug is dispersed as discrete solid particles within the polymer. A similar type of device to the dispersed matrix is the porous matrix where drug is also dispersed within the polymer at a concentration greater than its saturation solubility, however, in this type of device the drug particles form contiguous channels through the matrix. A final example of a matrix device are surface treated devices where a core of drug dispersed in polymer is surrounded by a surface layer which is of lower permeability to drug than the core. Reviews of the equations applicable to these systems and others can be found [15,30,62,64], however they are all based on the equation developed by Higuchi to describe the release of drugs

from an ointment base [63]. A number of assumptions are made in the derivation of these equations [30,65], for example, it is assumed that the diffusion coefficient remains constant during release and that sink conditions prevail in the release medium which will not always be the case.

Some of the cited advantages of matrix devices include their ease of manufacture, and also the fact that on accidental rupture there is less chance of a potentially dangerous release of drug occurring unlike reservoir type devices [30]. One of the disadvantages of matrix devices is that the release rate is time dependent [30], however it is possible to get near zero-order release in certain cases. One approach is to use surface treated devices [30], another method involves the use of hemispherical devices coated with an impermeable coat except for an exposed cavity in the centre face of the device [66]. Zero-order release may also occur from matrix devices if the boundary layer around the matrix becomes the rate limiting step in drug release, which would occur for instance when the drug is more soluble in the device than in the dissolution medium, or when the diffusion coefficient in the boundary layer is very low [30,60].

Matrix devices can be engineered to enable the release of macromolecules such as insulin [43]. This has been demonstrated by the solvent casting of normally impermeable polymers such as ethylene-vinyl acetate copolymer in volatile solvents along with powdered macromolecules [67,68]. This results in the formation of a series of interconnecting channels large enough to permit macromolecular release over a prolonged period.

Another type of matrix device is the so called swelling-controlled system where diffusion may not be the sole mechanism controlling release [15,69,70,71]. In this device drug is dispersed in a glassy polymer matrix, where no diffusion can occur in the solid phase. When a solvent such as water penetrates however the glass transition temperature is lowered and the swelling polymer becomes rubbery and allows drug diffusion outwards. Zero-order release may be obtained from this type of device.

1.3.3 Biodegradable Polymer Systems

Biodegradable devices are constant delivery type devices which employ the use of bioerodible polymers [5,72,73]. Polymer bioerosion can be defined as the conversion of a water insoluble material to one that is water soluble, this can be accomplished by hydrolysis, oxidation or other mechanisms of cleavage under enzymatic action [73]. The most obvious application for this type of device is as an implant. The mathematical treatment of the drug release from biodegradable matrix devices may be complex, since more than one mechanism can occur, such as the concurrent diffusion of drug out of the polymer matrix and release due to polymer degradation [5]. Other processes such as swelling may also occur which will further complicate any mathematical treatment of release kinetics [72,74]. The rates of polymer biodegradation can be varied, for example by copolymerization and by careful selection of polymer weight distribution [74,75]. Bioerosion may be classified according to three mechanisms [73], however in practice erosion is often a combination of these mechanisms. Type I erosion occurs when water-soluble polymers that have been insolubilized by covalent crosslinking

undergo hydrolysis at either the crosslinks (Type IA erosion) or at the water-soluble backbone (Type IB erosion). Type II erosion occurs when water-insoluble macromolecules become water-soluble as a result of either hydrolysis, ionization or protonation of pendent groups. Type III erosion occurs when high molecular weight water-insoluble macromolecules are converted to small water-soluble molecules by hydrolytic cleavage of labile bonds in the polymer backbone. The actual bioerosion process can be further defined as being either homogeneous or heterogeneous [76]. Heterogeneous erosion occurs only at the polymer surface and is sometimes termed surface erosion. Homogeneous erosion occurs throughout the bulk of the polymer matrix.

At least two approaches have been used in the development of implants based on biodegradable polymers [74,77]. The first is based on a reservoir device (section 1.3.1) where a drug reservoir is surrounded by a biodegradable rate controlling membrane. In order for the device to achieve zero-order drug release under diffusion control, significant bioerosion of the rate controlling membrane should not occur until the device has been depleted of drug. The second approach involves simply dispersing the drug in the biodegradable polymer matrix, from which drug is released as the result of bioerosion. Several reviews are available on release mechanisms from biodegradable devices and the various materials that have been examined, including polyesters and polyamides [3,74,76]. Many of the biomaterials used have been selected on the basis of their biocompatibility, furthermore these materials have tended to be hydrophilic and as a result homogeneous erosion may occur which tends to make the release process difficult to predict [15]. Zero-order release is theoretically possible from biodegradable devices if

heterogeneous erosion is the only factor controlling drug release, and if the geometry used for the device is a slab [72]. In order to achieve zero-order release hydrophobic polyanhydrides have been investigated as potential biomaterials [78,79], and have recently been used clinically in the treatment of glioblastoma multiforme [80].

The advantages of biodegradable devices include those that polymer matrix devices possess (section 1.3.2), additionally, a major advantage is related to their use as implants since there is no need to remove the implant on exhaustion of the device, also adverse tissue reactions caused by foreign implants are less likely than a normal matrix device [5]. The disadvantages associated with the use of biodegradable devices include potential problems with biocompatibility of the non degraded polymer, the possible need for surgical implantation, and also the possible production of potentially toxic or irritant degradation products [5,72].

A potential type of delivery device related to biodegradable devices are pendent chain systems [25,81]. In these types of system, a drug is chemically bound to a polymer backbone (which may or may not be biodegradable) and is released by hydrolytic or enzymatic cleavage. In order to attain zero-order release the cleavage of drug from the polymer should be the rate-limiting step.

1.3.4 Stimuli Sensitive Polymer Systems

The use of stimuli sensitive polymers has aroused great interest in recent years due to their potential application as materials in controlled drug delivery systems. These polymers may respond to stimuli such as temperature changes, pH changes or to the

presence of an enzyme or other specific chemical. The effect elicited by a stimulant may include a change in the permeability of the polymer or the release of an immobilized drug from the polymeric material. These materials would have obvious applications in many of the other types of device discussed in this chapter such as reservoir devices (section 1.3.1), matrix devices (section 1.3.2) and biodegradable devices (section 1.3.3).

A recent review on chemically self-regulated drug delivery devices summarises some of the systems under investigation, including those being examined for potential use in insulin therapy [82]. The use of thermally responsive polymers [83,84] and pH responsive delivery devices have been reported [85,86,87]. The advantage of these stimuli sensitive systems are that potentially self-contained responsive controlled delivery devices are feasible. Devices where the release rate may be modulated by external stimuli such as a magnetic field, ultrasound or an electrophoretic current are discussed elsewhere (section 1.3.7, section 1.3.8, section 1.3.9).

An example of a stimulus sensitive device has been reported for the release of insulin from a matrix device [88]. The device described consists of an ethylene-vinyl acetate copolymer containing immobilized glucose oxidase and trilysyl insulin. The presence of glucose results in the production of gluconic acid which reduces the pH of the polymer microenvironment. The decrease in pH results in an increase in the solubility of the insulin and an observed reversible increase in the release rate of insulin was found.

1.3.5 Osmotically Controlled Systems

Osmotically controlled systems, in their simplest forms, are a constant release type of device. The elementary osmotic pump consists of a water soluble core or reservoir containing drug surrounded by a semipermeable membrane, with a small delivery orifice of well defined size [89]. When placed in an aqueous medium, water diffuses into the device through the semipermeable membrane and forms a saturated solution of drug inside the device. The core imbibes water due to inequalities in osmotic pressure on either side of the semipermeable membrane. For a system of constant internal volume the device will deliver a volume of saturated drug solution over a period of time equal to the solvent volume uptake. The rate of solute release is zero-order as long as excess solid remains in the core, but the rate declines parabolically towards zero once the drug concentration within the device falls below saturation. Experimentally obtained results for this type of device closely agree with the theoretically predicted release. The actual magnitude of the release rate will be determined by the permeability of the membrane to water and the osmotic properties of the core. The size of the orifice is critical and must satisfy certain requirements if zero-order release is to occur [89]. This type of device offers a number of advantages, firstly zero-order release is possible at higher delivery rates than possible with matrix or reservoir type devices. The release rate in osmotic devices is less likely to be affected by environmental conditions such as stationary layers or by the pH of the environment then previously discussed devices.

Osmosin, an indomethacin osmotic device, was the first clinically used osmotic device, but was withdrawn due to cases of

peptic ulceration, bleeding and perforation caused by adherence of the device to the gut wall resulting in local drug release [1]. Reviews of the various developments in the use and design of osmotic pumps can be found [1,4,23,90,91]. The use of stimuli sensitive polymers (section 1.3.4) has resulted in the development of potentially self-regulating osmotic pumps [86].

1.3.6 Infusion Pumps

Mechanical infusion pumps may be considered as either constant or controlled type delivery systems depending on their complexity. Three types of infusion pump for drug delivery can be distinguished, external devices, implantable devices and implantable devices with a specific sensor [92]. The latter system offers the opportunity for feedback control. Infusion pumps offer a number of advantages [25,93,94]. Delivery of drugs that are difficult to absorb or inactivated when ingested can be attained using infusion pumps, also drugs with very short biological half lives or complex dosage regimes may be delivered by infusion. The release from infusion pumps will in general be independent of the drugs physical, chemical and diffusive properties. These devices may also be designed to deliver drugs to specific sites or directly into the bloodstream. An advantage of internal over external pumps is the reduced risk of infection which is associated with chronic percutaneous access sites. The advantages of infusion pumps over other types of delivery device are that replenishment of the device may be a simple refill, also these systems allow the administration of large volumes of fluid and multiple drug therapies. The main disadvantages [25,93] of this type of device are cost, lack of portability for external pumps, and

problems with biocompatibility. Drugs to be administered by infusion pumps must also be water soluble and hydrolytically stable. A major risk is the consequence of mechanical failure, which includes dumping of the drug reservoir and the clogging of pumps. The application of these systems to a number of medical conditions have been described in several reviews relating to their use in heart failure, diabetes and cancer therapy [1,93-99].

1.3.7 Magnetically Modulated Systems

There have been two main divisions in the application of magnetism in drug delivery. One approach to using magnetism has involved drug targeting : drug either attached to or formulated with a magnetic material is localized at an area of the body by application of an external magnetic field [100,101]. Reported results from animal experiments are very promising, and a study using vindesine sulphate formulated into albumin microspheres containing magnetite demonstrates the potential of this method of targeting [100]. In experiments using rats with tumour nodules, the magnetic microspheres were infused into the rats and a magnetic field positioned through the nodules for a period of 30 minutes after infusion. It was found that high doses, normally fatal, could be safely administered. The treatment effected using the magnetic microspheres was significantly more successful than control experiments. The results from this work showed that the therapeutic index of the drug could be increased, together with reduced side effects and greater efficacy.

The second approach to the use of magnetism has been based on modulating drug release rates from matrix devices, with the aim of

developing controlled release devices. One of the first reported studies adopting this approach to drug delivery was based on slabs of ethylene-vinyl acetate copolymer incorporating bovine serum albumin (BSA) and magnetic steel beads [102-105]. In aqueous medium the slabs were found to release a slow steady basal amount of BSA, however on exposure to an oscillating magnetic field, a significant increase in the rate of drug release was found over the basal release rate. The factors found to affect the delivery rate during application of a magnetic field included polymer formulation, magnetic field orientation, field strength and frequency. Tests using rabbit corneas indicated favourable biocompatibility of the magnetic matrix device. In vivo experiments using diabetic rats implanted subcutaneously with magnetic matrices containing insulin demonstrated significant decreases in glucose levels within twenty minutes of applying an oscillating magnetic field external to the rats. The mechanism by which these devices act is not fully understood, two mechanisms have been postulated. The first suggestion is that the oscillating beads alternately constrict and dilate the pores within the polymer thereby squeezing drug out. A second hypothesis is that new pores are temporarily created during exposure to the magnetic field. Recent studies with hydrophilic polymers incorporating ferrite microparticles have also demonstrated the potential usefulness of this type of device [106]. The advantage of a magnetically controlled implant device would be that modulated control would be possible externally, indeed it is envisaged that perhaps a device may be developed whereby a magnetic matrix device may be implanted subcutaneously in the wrist and the delivery rate modulated by a watch like device [68].

1.3.8 Ultrasonically Modulated Systems

Two different approaches have been adopted in the investigation of the potential utilisation of ultrasound as a method of drug delivery. One approach has been to try and enhance the transport of drugs through the skin using ultrasound, a method termed phonophoresis. The second approach has been based on the modulation of the drug delivery rates from polymeric devices using ultrasound.

1.3.8.1 Phonophoretic Delivery Systems

Phonophoresis as a method of enhancing the effects of drugs applied transdermally has been the subject of recent reviews [107,108]. The reviews cover some of the theories regarding the mechanisms of phonophoresis and the effect of phonophoresis on the skin, drugs and enzymes. Ultrasound was found to cause damage to mouse skin under certain conditions and also to inactivate certain enzymes, however one of the drugs which has received the most attention in phonophoretic studies, hydrocortisone, was found to be unaffected by ultrasound. Various theories have been proposed as to the mechanism of phonophoresis, including cavitation and microstreaming. The authors of the reviews conclude that phonophoresis may be a safe technique for enhancing drug administration and effectiveness in humans. Recently reported investigations found no statistically significant effect on the absorption of lignocaine, and a small but significant effect on the absorption of fluocinolone acetonide using phonophoresis, also many previous studies were criticised for having inadequate controls [109,110]. McElnay et al. [109,110] questioned whether ultrasound

alone may be as effective as in combination with a drug and mention the fact that ultrasound is used in physiotherapy in the treatment of inflammatory conditions, and suggest that ultrasound has intrinsic therapeutic properties. The advantages of phonophoretic delivery would be those offered by delivery using the transdermal route (section 1.5), with the addition that drug delivery may be modulated.

1.3.8.2 Ultrasonic Delivery Systems

The use of ultrasound as an external means of modulating delivery from polymeric devices has been reported, effects on the release from reservoir systems [44,111], matrix systems [111,112,113] and biodegradable systems [80,112,113,114] have been investigated with the aim of developing controlled drug delivery devices where the delivery rate may be externally modulated. One of the first reports on the use of ultrasound on a polymeric delivery device examined the effect of ultrasound on the release of 5-fluorouracil from both reservoir and matrix devices constructed of ethylene-vinyl alcohol copolymer [111]. It was reported that the drug delivery rate could be increased significantly by irradiating with ultrasound, the increase being reversible on cessation of the ultrasound. The mechanism of action was not certain but it was speculated that an increase in temperature may facilitate diffusion. It was found that blood glucose levels could be significantly decreased by irradiating externally a subcutaneous reservoir device containing insulin which had been implanted in rats [44]. Studies investigating the mechanisms by which ultrasound increased the solute transport through membranes looked at a number of effects [114,115]. The effects of ultrasound on stagnant diffusion layers, interfacial temperatures, membrane

integrity and diffusant stability were investigated and it was found that none of these factors alone could explain the observed increases in permeability due to ultrasound. A study on the effect of ultrasound on the release of drugs from biodegradable polyanhydrides found that significant and rapid increases were found in the drug release rate upon exposure to ultrasound [80,112,114], the effects were reversible on cessation of exposure to the ultrasound. The increase in release rate was found to be related to the intensity of the ultrasound, the actual increase however could not be solely explained by an increase in the rate of bioerosion [116]. Proposed mechanisms for the effects of ultrasound were cavitation and acoustic streaming [114]. Experiments performed on a biodegradable polyanhydride matrix containing insulin subcutaneously implanted in rats indicated that the exposure of an external ultrasound source caused no damage to skin, also no degradation of insulin occurred on exposure to ultrasound [113]. The potential advantages of an ultrasound modulated system include the fact that no additional substances such as magnetic beads or enzymes need to be added to the polymer and drug device in order to achieve modulated drug delivery [113].

1.3.9 Electrophoretically Modulated Systems

The principle of electrophoresis has been utilised in the development of controlled drug delivery devices. Two different approaches have been adopted, the first involving the delivery of ionic drugs into the body by the use of an electric field, a method termed iontophoresis [117]. The second method involves using an electric field to modulate the delivery of ionic drugs from reservoir

type devices. Strictly speaking the term ionophoresis should be used for these systems rather than electrophoresis as the latter term is used to describe the migration of charged colloidal particles such as proteins in an electric field, whereas ionophoresis is more correctly applied to the migration of charged ions or molecules, however the term electrophoresis is generally used to encompass the migration of any charged particle [118]. The theoretical aspects of electrophoresis are discussed below in section 1.4.

1.3.9.1 Iontophoretic Delivery Systems

The facilitated permeation of drugs through the stratum corneum of the skin by iontophoresis has been the subject of a number of recent reviews [108,117,119]. The components required for iontophoresis consist of a drug reservoir, one delivery electrode, and one current returning electrode, both of which are attached to the skin, and an electrical power source in between them. As a consequence of the high resistance in the stratum corneum, the current flows through the skin beneath one electrode, through the body's interstitial fluids, and back through the skin below the other electrode. The flow of current between the electrodes may result in an appropriately charged molecule migrating into the skin, however the current carried under the skin is probably carried predominantly by other more mobile physiological ions. Once the drug penetrates the skin it is available for systemic distribution [108]. Iontophoretic transport through the skin is thought to be via the skin appendages [108,117,119,120,121]. Iontophoresis has been shown to enhance the transport of uncharged molecules as well as charged molecules, this is thought to be a result of current induced convective flow

[119,121,122]. A recently derived expression for the iontophoretic transport of ions across the skin (section 1.4.3) was applied to the transport of etidronate disodium across pig skin [123]. The derived expression was found to be in good agreement with experimental findings up to voltages of 0.25 V, however beyond this the relationship broke down. It was thought that this may have been due to larger voltages altering the properties of the skin, an effect which has been reported by some workers [120,124].

The factors affecting iontophoretic drug delivery include the charge on the drug, the conductivity of the drug, ionic strength, drug concentration and pH [108,117,119,123-126]. Reviews of the large number of drugs that have been successfully delivered by iontophoresis can be found [108,117,119], including the enhanced transport of macromolecules such as insulin.

Iontophoresis as a method of drug delivery offers a number of potential advantages including those offered by the transdermal route of delivery (section 1.5). In addition iontophoresis may potentially be used to deliver drugs in a controlled and modulated manner [117]. The disadvantages of iontophoresis include problems with regards to safety, such as burns, electric shocks and skin irritation [108,117,119].

1.3.9.2 Electrophoretic Delivery Systems

There have been a number of recent reports on the development of an electrophoretically modulated drug delivery device [127-130]. This type of device is based on a polymeric reservoir device (section 1.3.1), with the addition of a pair of electrodes on either side of the rate controlling polymer film, as shown in figure 1.1. The

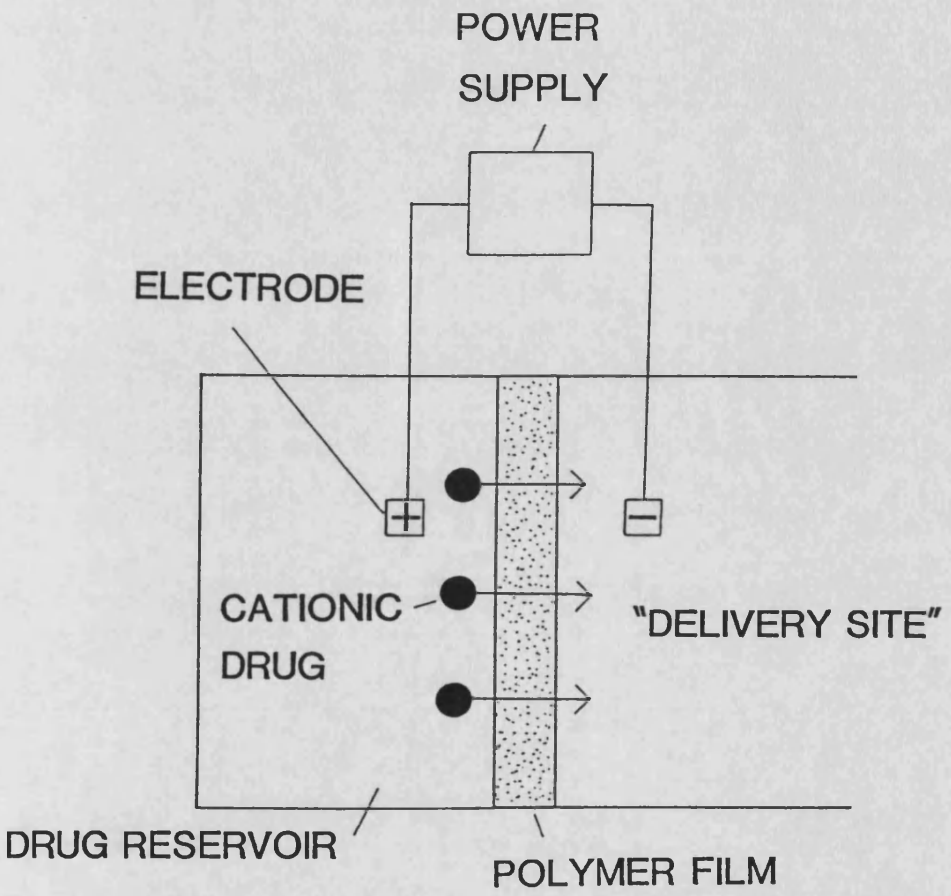


Figure 1.1. Diagrammatic representation of an electrophoretically modulated drug delivery device.

principle of the device is that the delivery rate of ionised drugs may be controlled. In the absence of an electric field a basal level of drug release will occur which may be modulated in a controlled and predictable manner by altering the magnitude of the electric field between the electrodes. Two approaches have been made to controlling the drug delivery rate, one involves the use of constant voltage electrophoresis, the other using constant current electrophoresis.

One of the first reported studies on an electrophoretic delivery system described the transport of a number of drugs, including a macromolecule (insulin), across discs of polyacrylamide and the effect of applying an electric field on the transport of these drugs through the polyacrylamide [127]. The system was characterized and several factors affecting migration examined. The author concluded that controlled voltage polyacrylamide electrophoresis appeared to be a promising means of providing drug release, though problems with reproducibility were found, which were thought to be due to changes in electrical resistance within the system.

A pencil-shaped electrophoretic delivery system has recently been reported for the transdermal application of diphenhydramine HCl [129]. The device uses a constant voltage supply and an agar gel drug carrier. It was reported that the release from the device could be varied over short periods (5 minutes) by varying the voltage, however very little information was given on factors affecting release. The same author also reports on the development of an electrophoretic capsule device for oral use [130].

The use of constant current electrophoresis as a means of controlled drug delivery has recently been reported [128]. The

authors examined an in vitro system based on polyacrylamide gel discs, and examined some factors affecting the release of bovine serum albumin. It was concluded that an electrophoretically controlled system shows potential in the delivery of charged molecules into a medium at constant, controlled and modulated rates.

There are a number of envisaged applications of an electrophoretic delivery device, one application is as a transdermal delivery device. Another envisaged application is as an implant system, where a device may be programmed to deliver a drug at a predetermined rate, or alternatively act as a feedback system where the drug output may be varied in response to a patients need. An example of a feedback system might consist of an insulin containing device which is refillable via a septum. The insulin output of the device could be controlled by a glucose sensor. The potential advantages of an electrophoretic delivery device included achieving full control over drug release which may be obtained by varying either the voltage or current across the device. The disadvantages of this type of device include the cost of producing a device, and biocompatibility problems associated with implant devices.

1.4 Electrophoresis

The theoretical treatment of the movement of small ions and colloidal particles under the influence of an electric field will differ, since unlike simple ions the charge carried by a colloidal particle is not a constant known quantity, and the behaviour of a colloidal particle will be greatly modified by the surrounding electric double layer [131].

1.4.1 Ionic Migration [132-136]

Many drugs are ionic in nature and in aqueous solution will dissociate into small ions which are free to carry electric currents when placed in an electric field. The ease with which a current is passed will depend on the conductivity of the electrolyte, which depends on the number of ions in a given volume of solution and the interaction between them. If electrolytes in solution were fully dissociated and no interaction existed between the ions then conductivity would be proportional to concentration, however the relationship is not so straightforward. It is often convenient therefore to express the conductivity of an electrolyte solution in terms of molar conductivity :

$$\Lambda = \frac{k}{C}$$

Λ = Molar Conductivity (Ω^{-1} m^2 mol^{-1})

k = Conductivity (Ω^{-1} m^{-1})

C = Concentration of Electrolyte (mol m^{-3})

The variation in molar conductivity with concentration will depend on whether the electrolyte dissociates completely in solution (a strong electrolyte) or only partly (a weak electrolyte). For strong electrolytes it is found that at low concentrations molar conductivity is a linear function of the square root of concentration, as described by the Onsager equation :

$$\Lambda = \Lambda_o - (A + B\Lambda_o).C^{1/2}$$

Λ_o= Molar Conductivity at Infinite Dilution (Ω^{-1} m^2 mol^{-1})

A = Constant

B = Constant

The Onsager equation describes the effects of ion-ion interactions on conductance in terms of the relaxation and electrophoretic effects, and is based on the Debye-Huckel theory which describes the ionic atmosphere around an ion. These effects account for the fact that as the chemical concentration of an electrolyte is increased the effective concentration will not increase proportionally. The electrophoretic effect arises when an ion moves through a viscous medium and drags with it the solution in its immediate vicinity. Neighbouring ions have to move with or against the stream depending on their charge. The relaxation effect is a result of distortion of the symmetrical distribution of the ion cloud around any given ion. At equilibrium the ionic atmosphere is, on a time average, distributed with spherical symmetry and therefore exerts no resultant force on the central ion. If the central ion moves to an off centre position it experiences a restoring force, which however rapidly dies away as the atmosphere is rearranged.

In the case of weak electrolytes the relationship between molar conductivity and concentration is more complex since the degree of dissociation is concentration dependent, the degree of dissociation (α) decreasing with increasing concentration. A modified form of the Onsager equation accounts for this :

$$\Lambda = [\Lambda_o - (A + B\Lambda_o)(\alpha C)^{1/2}]\alpha$$

At infinite dilutions, it is assumed that there are no forces of attraction between ions and the dissociation of all electrolytes is complete, thus the motion of an ion is solely determined by its interaction with surrounding solvent molecules. Kohlrausch's law of the independent migration of ions states that the molar conductivity for an electrolyte at infinite dilution is the sum of the ionic

conductivities for each type of ion, $\lambda_{\pm}$:

$$\Lambda_o = \lambda_+ + \lambda_-$$

An ion placed in an electric field will tend to move with a velocity proportional to the field strength. The electric mobility of an ion is defined as the velocity of motion of the ion divided by the electric field strength :

$$u_i = \frac{v}{E} = \frac{Q}{6\pi\eta r}$$

u_i = Mobility of Ion ($m^2\ s^{-1}\ V^{-1}$)

v = Velocity of Ion ($m\ s^{-1}$)

E = Electric Field Strength ($V\ m^{-1}$)

Q = Charge on Ion (C)

η = Viscosity of medium ($kg\ m^{-1}\ s^{-1}$)

r = Radius of the Ion (m)

The molar conductivity of an electrolyte may be related to the mobilities of individual ions ($u_{\pm}$) in the electrolyte as follows :

$$\Lambda = (u_+ + u_-).F$$

F = Faraday's Constant

The fraction of the total current carried by a particular type of ion is termed the transport number (t_i) of that ion :

$$t_i = \frac{u_i}{u_+ + u_-}$$

1.4.2 Colloid Migration [131,133-137]

Electrophoresis, the movement of a charged surface relative to a stationary liquid by an electric field, is one of four electrokinetic effects that occur across a solid-liquid interface due to differences in electric potential. The other three electrokinetic effects are electro-osmosis, streaming potential and sedimentation potential. Electro-osmosis is the movement of liquid relative to a stationary

charged surface by an applied electric field. The streaming potential is the potential difference created when a liquid is made to flow along a stationary charged surface. The sedimentation potential is the electric field created when charged particles move relative to a stationary liquid.

A colloidal particle such as a protein placed in water, may obtain a charge from either ionisation of surface groups or adsorption of ions which confer their charge onto the surface of the particle. This surface charge influences the distribution of ions in the medium, resulting in the formation of an electric double layer. The charged particle will attract a layer of counter-ions. If for example the particle has a negative charge, then this will result in the particle having a fixed layer of positive ions attracted electrically to the surface of the particle, this layer of ions being known as the Stern Layer. The electric potential across the Stern Layer decreases rapidly and linearly, the surface of this layer is the Stern Plane. Beyond the Stern Plane a second diffuse layer exists which contains an excess of loosely held positive ions, the surplus decreasing with distance from the particle. The electric potential decays exponentially across this diffuse layer. When a colloidal particle moves, the Stern Layer moves with it together with some solvent molecules in the diffuse layer, the outer boundary of this unit, the effective surface of the particle, is known as the Shear Plane. The zeta potential (ζ) is the potential at the Shear Plane, and it is this potential that determines the behaviour of a colloid particle in an electric field. The zeta potential is experimentally indistinguishable from the Stern Layer potential and is usually assumed to be the same. This will not be correct at high electrolyte

concentrations when the diffuse layer is compressed. The zeta potential can be related to electrophoretic mobilities, a number of simplified relationships may be used, the type of relationship applied will be determined by the dimensionless quantity ka, which is the ratio of radius of curvature to double layer thickness. If ka is small the particle may be treated as a point charge, when ka is large then the double layer may be treated as a flat surface. A number of equations have been used to describe the mobility of colloids including the Huckel equation (small ka) and the Smoluchowski equation (large ka). A more general relationship is the Henry equation, which for a non-conducting particle can be written as :

$$u = \frac{\epsilon \zeta}{6\pi\eta} f(ka)$$

ϵ = Permittivity of the Medium ($C\ V^{-1}\ m^{-1}$)

The value of f(ka) varies between 1.0 for a small ka (Huckel equation) and 1.5 for a large ka (Smoluchowski equation). The Henry equation takes into account the electrophoretic retardation effect, however it makes a number of assumptions, such as the viscosity and permittivity being constant throughout the mobile part of the double layer. The Henry equation also does not take into account the relaxation effect and surface conductance effects in the vicinity of the charged surface. The electrical conductance in the diffuse part of the double layer is greater than that in the electrolyte medium due to the distribution of ions in this region, surface conductance may affect the electric field distribution near the surface of the charged particle, particularly if ka is not small, and so influence the electrokinetic behaviour of the particle.

1.4.3 Transport of Ions through Homogeneous Membranes

The study of diffusing ions under the influence of an electric field has been of importance in the fields of electrochemistry [133] and physiology [138]. Keister and Kasting have recently developed a model to describe the iontophoretic phenomenon of an ionised drug diffusing from a reservoir through an uncharged homogeneous macroscopic membrane under the influence of an electric field [139]. The derived equation is based on the Nernst-Planck equation which separates the flux of an ion diffusing under the influence of an electric field into its diffusion and migration components as shown below [133] :

$$J = -D \frac{dC}{dx} + \frac{DzeEC}{kT}$$

where J is the drug flux, D is the diffusion coefficient, C the concentration of ions with valency z and charge e, E the electric field strength, k Boltzmann's constant, and T the absolute temperature. The boundary conditions of the model were as follows: at time t=0 there is no drug in either the membrane or in the receptor solution, the solutions on both sides of the membrane are well stirred and sink conditions prevail in the receptor. The derived equation is based on a number of assumptions, such as the electrical field being constant, which the authors justified on the basis that the primary current carrying ions are smaller ions such as Na^+ and Cl^-, and that these will be distributed uniformly throughout the membrane, and that there will be at most very low net charge densities throughout the membrane. The model is however simplistic and limited, since the influence on flux as a result of interactions between ions is not considered, and would thus only be applicable at low ionic strengths [117,140]. The relationships that were derived

from this model describe the flux enhancement ratio produced by an applied voltage, J(v)/J(0), once steady state has been attained :

$$\frac{J(v)}{J(0)} = \frac{v}{1 - e^{-v}}$$

where v is defined as :

$$v = \frac{zeV}{kT}$$

and the ratio of voltage enhanced lag time to the passive lag time, $T_L(v)/T_L(0)$, is given by :

$$\frac{T_L(v)}{T_L(0)} = \frac{6.[v.\coth(v/2)-2]}{v^2}$$

The model predicts that the application of a uniform electric field across a uniform membrane leads to ion flux enhancements proportional to v and to a reduction in lag times. The model also predicts that it should be possible to not only enhance flow but to also actually shut down transport by reversal of polarity.

1.5 Transdermal Drug Delivery

Drug delivery systems may be classified by the route of delivery, such as implantable delivery systems, oral delivery systems, transdermal delivery systems, and other non-invasive delivery systems. The transdermal route has been extensively researched as a potential route for drug delivery due to a number of potential advantages which this route offers compared with the oral route of delivery, which is currently the most popular method of delivery. There are many recent reviews dealing with transdermal drug delivery [26,141-151]. Some of the problems with drug absorption from the oral route include variability of gastro-intestinal motility and transit times, the exposure of drugs to the first-pass effect, stability of the drug in the gastro-intestinal tract, and the varying

and variable absorption capabilities in different parts of the gastro-intestinal tract. All of these factors will influence the bioavailability of a drug and results in the oral route offering limited potential for constant and controlled drug delivery devices [24,135,152,153,154]. The transdermal route of delivery can overcome some of the variables which affect gastro-intestinal absorption, other advantages include an improvement in patient compliance, and the fact that treatment may be easily terminated [1,135,141]. The transdermal route may also be a suitable route for constant and controlled drug delivery devices, however the latter type of device would have to overcome the difficulty of the long lag times generally involved with transdermal transport [141]. The transdermal route does however have a number of significant limitations. One of the main functions of the skin is to act as a barrier which is generally very effective and has caused transdermal delivery to be limited to extremely potent drugs. However, research into enhancing drug delivery by either chemical methods (section 1.5.2) or physical methods (section 1.3.8.1, section 1.3.9.1) has shown that the effectiveness of this barrier function may be reduced locally [26,141,142]. Another problem with transdermal delivery is possible irritation or allergic reaction to either the drug or delivery device [142]. Drug degradation either on or in the skin may also be a potential problem, this may occur as a result of either the presence of enzymes in the skin or micro-organisms on the skin surface [142,155,156].

1.5.1 Transport of Drugs through Skin

The human skin may be considered as consisting of two distinct layers (figure 1.2), the stratified avascular cellular epidermis and the underlying dermis of connective tissue. A fatty subcutaneous layer resides beneath the dermis. Human skin is interspersed with appendages, such as hair follicles, sebaceous glands and sweat glands, which pass through pores in the epidermis to reach the surface. The appendages however only represent a small fractional surface area of the skin [157]. The epidermis may be differentiated into two layers, the stratum corneum, the uppermost layer which is approximately 10 μm in thickness, and the viable epidermis, which may vary in thickness between 60 and 800 μm. The stratum corneum is composed of flattened keratinized dead epidermal cells, stacked in units consisting of highly organized vertical columns. In general body areas the stratum corneum is 10 to 15 cell layers in thickness, whereas the thickness of the dermis may vary between 3 and 5 mm. A more detailed consideration of the skin structure may be found elsewhere [26,158].

It has been suggested that the stratum corneum may be represented as a wall-like structure built up of protein bricks (the corneocytes which are mainly composed of keratin) and joined by lipid mortar [159,160]. The lipids within the stratum corneum are thought to be structured as bilayers [161,162]. It is the stratum corneum that is thought to be the rate-limiting barrier to the transport of most drugs through the skin [158,163], except for very lipophilic drugs where the aqueous dermal layer may provide significant hindrance to transport [142,163]. The transport of drugs across the stratum corneum may occur either via the appendageal pathway or the

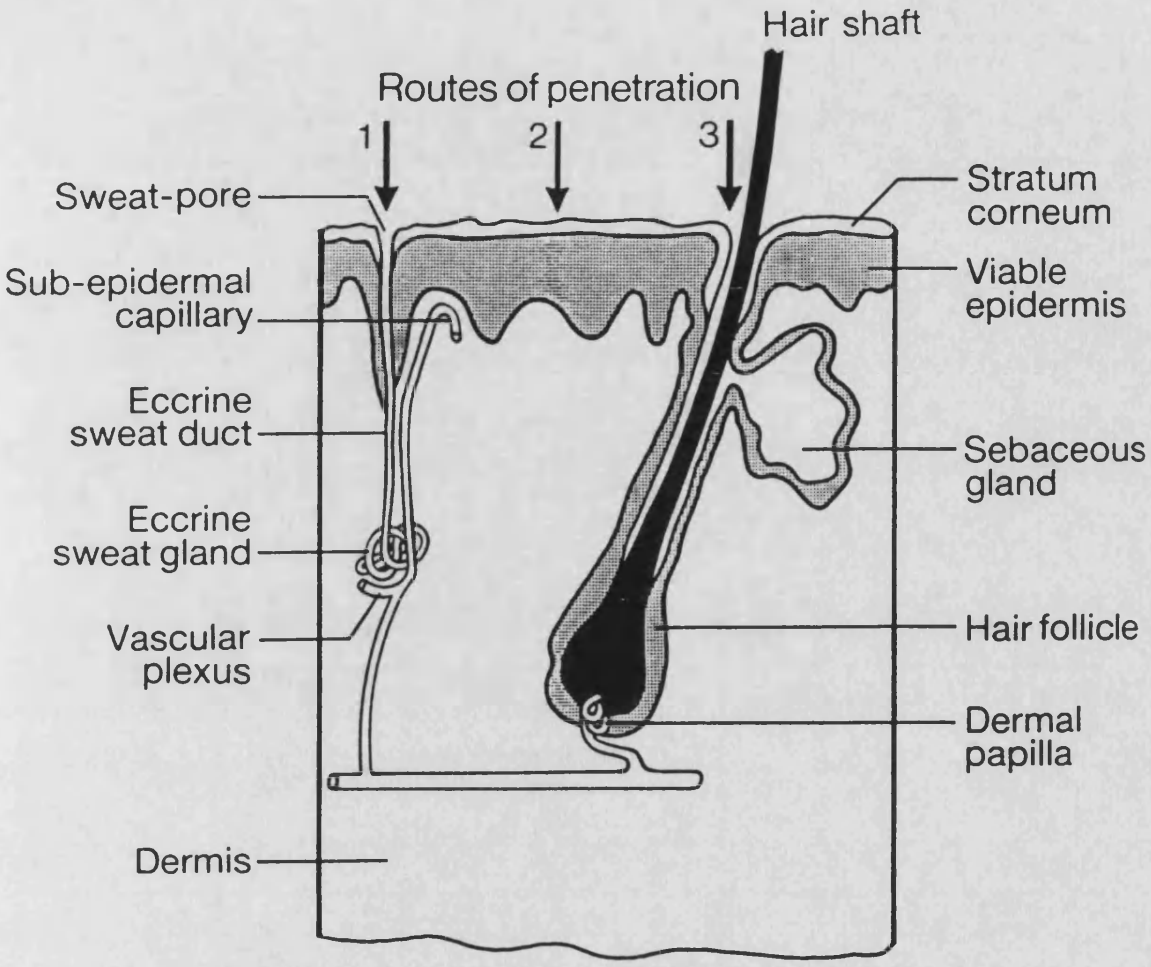


Figure 1.2. Diagrammatic structure of human skin with three possible routes for drug transport - (1) via the sweat glands, (2) across the intact stratum corneum, (3) via the hair follicles [26].

transepidermal pathway [26,141,164,157]. The mechanism of transport is still not precisely known, though it is believed that the transepidermal route is the main transport route for most drugs, however the appendageal pathway may be important for short diffusional times and for large polar molecules [142,163]. Transepidermal transport may be either transcellular or intercellular, the relative importance of these routes for a particular drug will depend amongst other things on the drugs solubility, partitioning behaviour with the various phases, and diffusivity within these phases. In general it is thought that the transcellular route predominates for polar drugs, and that as polarity decreases the intercellular route becomes increasingly predominant. Whatever the predominant transepidermal route, it is generally acknowledged that the stratum corneum lipids provide a significant part of the barrier function of the skin [163,165,166]. It is usually found that lipid-soluble drugs penetrate the skin better than water-soluble drugs, and for drugs which can exist as both ionised and unionised species, it is generally the unionised species that are more permeable [160,167]. It has also been demonstrated that the permeation of charged species may be increased by increasing the lipophilicity of the ionised permeant through ion-pair formation [168,169].

The transport process for a drug permeating the skin is quite complex [26,141,170], once delivered to the skin surface the drug must partition into the stratum corneum and diffuse through this impermeable barrier. The drug may interact with binding sites, free drug eventually reaching the viable epidermis and the drug must partition into this water rich layer. The drug must then diffuse in

the viable epidermis and partition into the dermis where a combination of events may occur such as the drug forming a depot, the drug being degraded or the drug partitioning into a blood capillary for systemic transport. Reviews on the many factors that can affect the transport of drugs through skin are available [26,147-150,171]. Some factors that will affect drug transport include skin age, skin condition, absorption variations with body site, individual variations, skin hydration, temperature, pH, the solubility characteristics of a drug and the partition coefficient of a drug.

1.5.2 Penetration Enhancers

Ideally, transdermal drug delivery devices should deliver drugs at a controlling rate well below the maximum rate that the skin can accept in order for the delivery device to be genuinely rate controlling and safe [108,141,170]. Unfortunately the low permeability of the skin makes this difficult to achieve in practice. However, using penetration enhancers it may be possible to increase the permeability of the skin, and thus increase the range of drugs which may be delivered transdermally. An ideal penetration enhancer should possess a number of properties [26], such as being pharmacologically inert, non-toxic, non-irritating, odourless, colourless and tasteless. In addition the enhancer should be specific in action, act immediately and its action should be reversible.

A number of compounds have been examined as potential penetration enhancers including dimethyl sulphoxide (DMSO), pyrrolidones, and a number of surfactants. These and other compounds have been the subject of recent reviews [26,108,172,173,174]. 1-Dodecylhexahydro-2H-azepin-2-one (Azone) is a relatively new

penetration enhancer which has received a great deal of attention, studies having demonstrated the enhanced penetration of both hydrophobic and hydrophilic drugs in the presence of Azone [175-183]. Reported studies indicate that Azone shows no indications of toxicity or irritation, additionally Azone appears to be poorly absorbed, and what little is absorbed is rapidly cleared by the kidneys. The effects of Azone on the permeability of the skin also appear to be reversible.

The mechanism by which penetration enhancers increase the transport of drugs through the skin is still uncertain though they are believed to act at the stratum corneum. One theory, based on results from permeation and differential scanning calorimetry studies, is that enhancers affect the nature of lipid bilayers in the stratum corneum by disrupting the intercellular lipid structure from a state of relative order to disorder, and thus increase the fluidity in this region, permitting drugs to permeate more readily [163,178,184,185,186]. Enhancer molecules may interact at three main sites of the lipid bilayer structure, namely the polar heads of the lipids, within the aqueous regions between lipid head groups, and between the hydrophobic tails of the bilayers. An effect on the keratin fibrils and their associated water in the corneocytes may be the mechanism by which enhancers affect the intracellular route of drug transport. In addition to these effects some penetration enhancers may act by altering the partitioning properties of the stratum corneum. Support for this theory comes from studies using a fluorescent probe technique and electron spin resonance studies on model phospholipid membranes which also indicate that enhancers increase lipid fluidity [187,188].

1.5.3 In Vitro Methods for Studying Drug Transport through Skin

The majority of studies that have examined the transport of drugs through the skin have been based on in vitro experiments using excised skin, the assumption being that the stratum corneum is the rate-limiting step in drug transport, and that this dead horny layer maintains its barrier function in vitro. In vitro experiments have demonstrated the validity of this assumption [189]. Human skin is the most satisfactory type of skin for in vitro experiments, however availability may be a problem which has lead to the investigation of artificial membranes [26,190] and excised skin from a variety of animals such as the hairless mouse, pig and rat, however the results from using different types of skin should be viewed with care as skin morphology and drug transport will vary with species [26,191-194]. Even if human skin is used in experiments, data obtained usually suffers from considerable variability, even if a specific anatomic site is selected the permeability of skin can vary within a site for a given individual as well as varying between different individuals [26,195].

Hairless mouse skin has been reported to be a useful model for human skin, studies on the permeability of a series of alcohols indicated that the barrier properties of human skin and hairless mouse skin may be similar [196]. The results from a recent report however suggest that hydration damage does not make mouse skin suitable for long term studies [197]. It has also been recently reported that hairless mouse skin displays exaggerated responses to penetration enhancers compared to human skin [198,199]. These findings suggest that results from in vitro experiments using

hairless mouse skin should be treated with caution.

The transport of drugs through skin is usually measured by clamping the skin in a diffusion cell of some description and monitoring the delivery rate of drug from a donor compartment into a receptor solution. The choice of receptor solution may significantly affect the transport rate, particularly with lipophilic drugs [200]. Various types of diffusion cell have been used, however most are based on a similar basic design [201]. Two techniques have been adopted in studying drug transport, the infinite dose technique and the finite dose technique [202]. In the infinite dose technique the donor compartment contains a solution of the drug under study, and the build up of drug in the receptor is monitored during the course of an experiment. Generally the concentration in the donor remains constant during an experiment and the receptor acts as an effective sink, thus after a lag period steady state diffusion would be expected (appendix 1). If a plot of amount of drug in the receptor compartment against time is plotted, the slope of the linear part of the plot (steady state region) will give a measure of steady state flux. The transport behaviour of drugs in the skin is usually described by its permeability, which can be related to flux as follows:

$$\frac{dM}{dt} = PC'$$

$\frac{dM}{dt}$ = Flux (mol cm^{-2} s^{-1})

P = Permeability Coefficient (cm s^{-1})

C' = Initial Concentration of Drug in Donor Compartment (mol cm^{-3})

where P is defined as:

$$P = \frac{KD}{h}$$

K = Partition Coefficient of the Solute between Membrane and Bathing Solution

D = Diffusion Coefficient ($cm^2\ s^{-1}$)

h = Membrane Thickness (cm)

The permeability coefficient is used since it may be difficult to measure the partition coefficient, also the measurement of membrane thickness may be difficult. The infinite dose technique has been criticised as not being a realistic model, one of the main objections being that both sides of the membrane are exposed to an aqueous medium thus excessively hydrating the skin [202]. Some workers prefer the use of the finite dose technique where the donor side of the skin (the epidermis) is exposed to ambient conditions and a fixed dose of drug is applied either by rubbing in, or applying in a volatile solvent. Using this technique steady state diffusion is not achieved, after a lag time the flux rises to a peak than falls as the drug concentration on the exposed epidermal surface decreases, thus permeability coefficients can not be calculated. The manner of application in the finite dose technique is thought to mimic more closely the way that a drug is applied clinically.

1.6 Poly(2-hydroxyethyl methacrylate) (PHEMA)

The use of polymers as materials for drug delivery systems has been the subject of a number of reviews [7-11,25,203-206]. PHEMA is a hydrogel that has been extensively researched as a material for biomedical applications since it was first introduced by Wichterle and Lim [207]. A hydrogel may be defined as a hydrophilic polymeric

material that swells in water, retaining a significant amount of water, but remaining insoluble [208]. The amount of water that equilibrated hydrogels may hold can range from 3% to greater than 90% depending on the hydrogel [209]. Interest in PHEMA stems from a number of favourable properties which this polymer possesses (see section 1.6.2), which has made it a candidate for use in drug delivery systems.

1.6.1 Preparation and Structure of PHEMA

PHEMA is an addition polymer produced by the free-radical polymerization of 2-hydroxyethyl methacrylate (HEMA). PHEMA hydrogels are usually polymerized in the presence of a crosslinking agent, the structure of PHEMA and PHEMA crosslinked with ethylene glycol dimethacrylate are shown in figure 1.3. Radicals may be generated by either chemical catalysts, UV radiation in the presence of a photosensitive chemical, or by ionizing radiation [60,209-212]. The actual polymerization process may be one of several methods such as bulk polymerization of the monomer with crosslinking agent, crosslinking of the polymer in solution, or simultaneous polymerization and crosslinking of a monomer with a crosslinking agent in solution [213]. The latter method is often preferred since polymerization may be achieved quickly at a low temperature, also the shape of the polymer may be easily determined since the starting materials are in liquid form. When HEMA is polymerized in solution with a crosslinker, water may be used as a solvent. If the water content is maintained below a certain value then optically transparent homogeneous hydrogels are formed, however if the water content is above this value then opaque and porous heterogeneous

a)

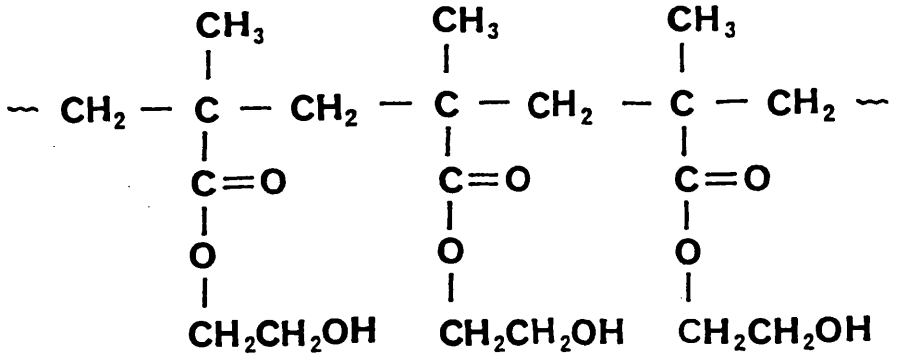
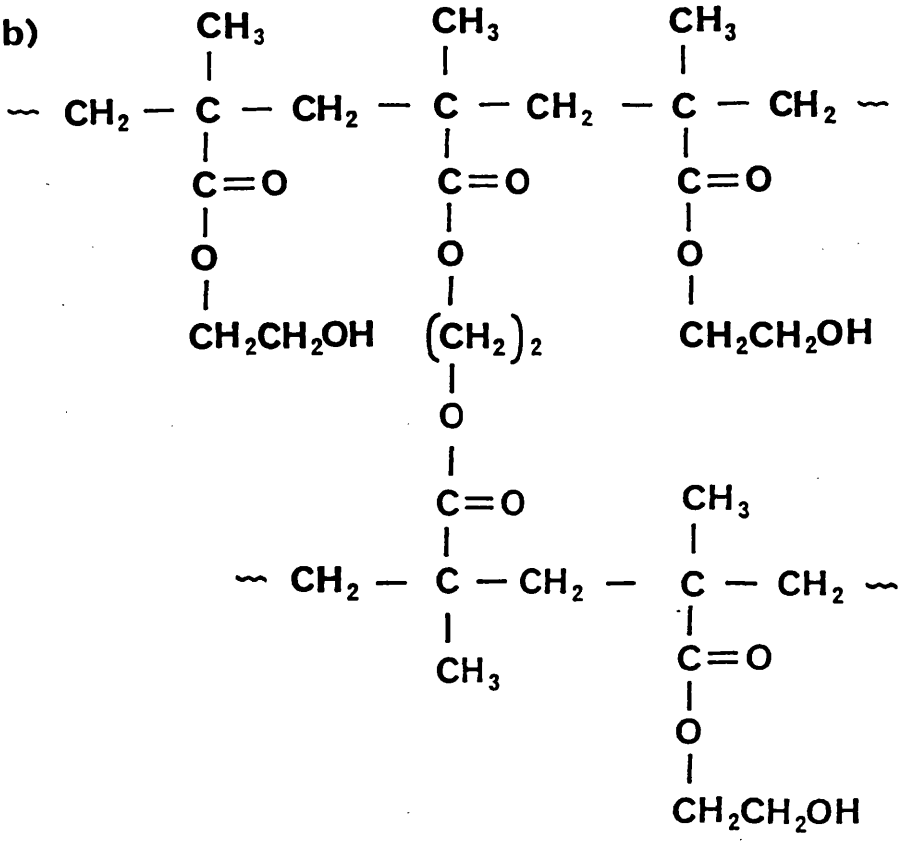


Figure 1.3. Structure of PHEMA (a) and PHEMA crosslinked with EGDMA (b).

b)



hydrogels are formed due to a phase separation [213,214]. The critical value at which this change occurs has been reported to be in the region of 40% to 43% [213,215,216].

In the absence of crosslinking agent PHEMA has been reported to still possess a significant crosslinking density due to physical chain entanglements and noncovalent interactions between the chains [217]. In addition to the 3-dimensional covalent network in PHEMA there is believed to be a secondary structure superimposed upon the covalent network, which is thought to explain some of the properties of PHEMA (section 1.6.2) such as the narrow range of swelling that PHEMA exhibits at low levels of crosslinking [213]. One of the first models proposed for this structure was that hydrophobic bonding between the α-methyl groups in PHEMA, and also possibly between the chain backbones, was responsible for holding together the secondary structure in an aqueous environment [218]. A more favoured model however is that the secondary structure consists of hydrogen bonds between pendent hydroxyl groups (and less likely pendent hydroxyl and carbonyl groups) which are stabilized by the steric exclusion of water molecules from the regions containing the bonds [219]. It was shown however that some hydrophobic bonding probably does occur within the polymer matrix, but is not the main cause of the secondary structure. It should be noted that these models are partly based on the interpretation of swelling effects that urea has on PHEMA, and that doubts have recently been cast on the relevance of these experiments. It has been reported that urea does not swell PHEMA directly, but that the decomposition of urea in solution occurs which results in an increase of pH in the polymer. This increase in pH is thought to ionise methacrylic acid, which is usually present as an

impurity in PHEMA, and that the swelling caused is a consequence of this ionisation and the resultant electrostatic interactions [220].

The equilibrium water content of PHEMA is found to be remarkably insensitive to the addition of low levels of crosslinker, the water content being approximately 40% [213]. It has been proposed that the water within PHEMA is structured at a molecular level within the network, there have been a number of theories as to the nature of this structuring. One model, the three state model, proposes that the water within PHEMA consists of bound, free and intermediate water [221]. Evidence for this model stems from a number of studies including proton pulse nuclear magnetic resonance (NMR), differential scanning calorimetry (DSC) and dilatometry [215,222,223]. The bulk water is the water fraction within the polymer with properties most like water in free aqueous solution. The bound water fraction is strongly associated with the polymer backbone and is thought to be orientated around hydrophilic groups. The intermediate water is also thought to be associated with the polymer backbone, but is not as strongly associated as the bound water. The bound and intermediate fractions have been termed Domain A, a region of water thought to be associated with the mechanical properties of the polymer backbone and relatively hydrophobic in nature. Domain B consists of free bulk water, the region of the polymer associated with hydrophilic properties [224]. The relative amounts of each type of water will vary depending on the total percentage of water within the hydrogel and the crosslinker content. Evidence from NMR and DSC studies indicate that as PHEMA imbibes water from the dry state the first step is the binding of water to hydrophilic sites (bound water), additional water is preferentially orientated around the bound water

and the polymer network structure as a secondary or tertiary hydration shell and any excess water is present as free water (bulk water) [223]. Thus if the water content of PHEMA is reduced below equilibrium the relative proportion of bound water increases at the expense of intermediate and bulk water, and at a concentration of about 30% water there is thought to be no bulk water present. An increase in crosslinker content was also reported to increase the proportion of bound water. A more recent model suggests that PHEMA containing equilibrium water contains virtually no bulk water. The model proposes that the water and polymer form a coherent, integral structure in which there is no place for solutes [225], and that the internal structure of PHEMA is best described as an elastic solution of polymer in water [226,227]. The authors suggest that since swollen PHEMA (of 40% water content) contains at most about five molecules of water per monomeric unit of PHEMA, that it is unlikely that separate regions of water can exist large enough to be free of any influence of the polymer. The model is based mainly on DSC, adiabatic calorimetry and swelling experiments. The model proposes that solute absorption is only possible if the solute can disrupt the integral structure which leads to increased water uptake and swelling. Alternatively, if a solute cannot enter the polymer structure significantly, a deswelling of the polymer will occur due to an osmotic effect. The authors of this model have not yet attempted to relate the proposed structure to the transport of solutes through PHEMA, however the three state model has been extensively used to explain the transport behaviour of both hydrophilic and hydrophobic solutes through PHEMA (section 1.6.3).

1.6.2 Properties of PHEMA

PHEMA has attracted much attention as a material for biomedical applications due to its physical and chemical properties. The literature on the properties and uses of PHEMA and its copolymers in drug delivery systems and other biomedical applications is extensive, however several reviews have been published [10,60,204,205,206,208, 228]. Some of the biomedical and pharmaceutical applications of PHEMA include the use of PHEMA in contact lenses, artificial tendons and intrauterine devices. Some of the polymers properties will be briefly reviewed in this section, however a more detailed consideration of the properties of PHEMA relevant to this work will be considered in other sections.

PHEMA has been reported to be a biocompatible material, a property which is thought to result partly from its high water content [60,207,229,230]. Biocompatibility is a general term used to describe a wide variety of biological responses, thus the compatibility of a material should be investigated with a view to the specific intended application of that material. PHEMA has been suggested as a biocompatible material due to a number of favourable properties. The high water content and soft rubbery consistency of these materials gives them a strong superficial resemblance to living tissue which may partly explain why PHEMA does not cause mechanical irritation when in contact with cells and tissue. The low interfacial tension found between the surface of PHEMA and aqueous solution minimises protein adsorption and possibly cell adhesion. In addition polymerization initiators, residual monomer and other impurities can easily be removed from PHEMA prior to use by washing, which reduces the likelihood of toxic reaction to the polymer. The ability of ions

and metabolites to diffuse through PHEMA has also been cited as an advantageous property. There have been studies indicating favourable biocompatibility of PHEMA implants in vivo [231], however it has also been reported that thick fibrous capsule formation around PHEMA implants occurs together with tumour production in rats [232].

PHEMA has been found to be resistant to acid hydrolysis and reaction with amines [233]. A polymer related to PHEMA, poly(diethyleneglycol methacrylate), was found to incur alkaline hydrolysis only at elevated temperatures and at a high pH [234]. The water content of PHEMA has been reported to be relatively insensitive to the addition of low levels of crosslinking agent [213], additionally the equilibrium swelling of PHEMA in water prepared by solution polymerization was found to be fairly constant regardless of the initial dilution of the monomer solution (providing the solvent concentration is not high enough to form heterogeneous PHEMA) [213]. PHEMA prepared by bulk polymerization will swell in water to a similar equilibrium water content as that prepared in aqueous solution [218]. Temperature has been reported to have a small effect on the equilibrium water content of PHEMA over the range 10 to 100°C [213].

The mechanical properties of PHEMA have also received some attention, and the relevant literature has been reviewed by Janacek [235].

1.6.3 Diffusion of Solutes in PHEMA

The three state model of water has been used as a basis for developing models to describe the transport behaviour of solutes in PHEMA. The diffusion of solutes through PHEMA depends on the amount

and type of crosslinking agent, the water organisation within the polymer and the size and nature of the solute (hydrophobic or hydrophilic). A number of investigators have examined solute transport through hydrogel membranes and several reviews of this work have been published [10,60,204,205,236]. In this section a selection of these studies will be briefly reviewed.

Yasuda et al. examined the permeation of hydrophilic solutes through a series of hydrogels of varying hydration utilizing the free volume theory [237,238,239]. It was assumed that the effective volume for solute diffusion corresponds to the free volume of the aqueous phase and that the solute diffuses through "fluctuating pores" by successive jumps through holes which are larger than the solute. The solute was also assumed to permeate through aqueous regions only and that any interactions with the polymer are minimal. The theory was found to be applicable at high values of hydration where a predicted linear dependence of the logarithm of the permeability on the reciprocal of the volume fraction of water in the swollen hydrogel was found to occur with the model solutes examined. The slope of these plots was found to be dependent on solute size. Wisniewski and Kim also applied the free volume theory to examine the effect of apparent solute molecular size on the diffusion through PHEMA membranes [240]. A range of water soluble molecules were examined in the molecular weight range 20-500 Dalton. It was found that for 1 mole% crosslinked PHEMA that the diffusion coefficient of these solutes decreased exponentially with increasing molecular size. The authors also implied that the permeation of these solutes probably occurs primarily via bulk water, however exceptionally high values for the partition coefficients of thiourea and sodium methotrexate

were found compared to the other solutes examined, and it was thought that these solutes may interact with the polymer chains.

Kim et al. examined the permeation of hydrophilic and hydrophobic solutes through PHEMA membranes [224,241]. It was reported that hydrophilic solute permeation, through PHEMA with a low crosslinker content, was consistent with a pore mechanism through domain B (section 1.6.1) composed of fluctuating pores created by the interpenetration of chains in the polymer network. The pore size is determined by the percentage of crosslinking agent which will in turn determine the equilibrium water content. The effect of solute molecular size on solute diffusion was described in terms of a free volume model at low crosslinker contents, at higher crosslinker contents however deviations from this model were found. The diffusion of hydrophobic solutes through low crosslinked PHEMA was also postulated as being predominantly by a pore mechanism, however at higher crosslinker contents, as the effective pore size decreases, a partitioning mechanism was thought to predominate where diffusion occurs between polymer segments via domain A water. The diffusion coefficients of hydrophobic solutes were found to be lower than those for hydrophilic solutes.

Lee et al. examined the transport of a series of amides across PHEMA crosslinked with tetraethylene glycol dimethacrylate (TEGDMA) and the effect of varying the amount of TEGDMA in the polymer [242]. They found that the permeability and diffusion coefficients decreased with an increase in crosslinker concentration for all the amides examined. It was proposed that as crosslinker content was increased the pore size in the polymer decreased and that the mechanism of diffusion changes from a pore mechanism to a partition mechanism.

Zentner et al. performed a similar experiment with the hydrophobic compound progesterone and found similar changes in diffusion coefficient as crosslinker content was increased [243]. The results were interpreted once again as a change in diffusion mechanism from predominantly a pore mechanism to a partition mechanism.

Collett et al. examined the effect of polymer hydration on the diffusion of salicylic acid through crosslinked PHEMA. [244,245]. It was found that a plot of log diffusion coefficient of salicylic acid against polymer concentration showed a sharp change of slope at a polymer concentration of 69%. The authors correlated this value to the hydration value at which no bulk water exists in PHEMA (about 30% [215]) and concluded that at a hydration value of 31% and greater, transport of salicylic acid occurred by a pore mechanism via the bulk water, however below 31% hydration, the small pore size and absence of bulk water resulted in a change in transport mechanism. The effect of hydration on effective crosslinking density (calculated from stress-strain data) was examined and showed a sharp upward displacement when the hydration corresponded to 31%. The authors concluded that intermolecular bonding increases in the absence of bulk water.

1.6.4 Interaction of PHEMA with Ions in Solution

The accumulation of a component in solution at an interface between phases is termed adsorption. It is a surface effect and two general types of adsorption may be distinguished, physical adsorption, in which the solute is bound through relatively weak forces such as van der Waals or hydrogen bonds, and secondly chemical adsorption which involves stronger valence forces [135]. Adsorption

from solution is usually expressed in the form of adsorption isotherms in which the uptake of solute per unit weight of adsorbent is plotted as a function of the concentration of solute remaining in solution at equilibrium. The shape of the isotherm has been used as a basis for the classification of solution isotherms [246], the various types of isotherm being associated with different adsorption mechanisms. Adsorption of solutes by polymers and factors affecting adsorption such as the solvent type, temperature, pH and ionic strength have been reviewed elsewhere [246,247,248].

PHEMA has been shown to non reversibly adsorb the cationic preservative chlorhexidine digluconate in solution, the sorption isotherm produced being a typical high affinity type isotherm (H1), which normally occurs when a solute has such a high affinity for the adsorbent that it is completely removed at low concentrations [246,249]. The interaction was found to occur to a much greater extent when chlorhexidine was in its ionised form. The nature of the interaction was suggested to be due to an interaction between the cationic chlorhexidine ion and negatively charged sites within the polymer. Studies with monofunctional biguanides (chlorhexidine being a bis biguanide) indicated that the extent of interaction with PHEMA increased with biguanide alkyl chain length, and together with other findings it was suggested that the ion-ion interaction between chlorhexidine and PHEMA is stabilized by van der Waals forces between the alkyl chain and the polymer backbone and perhaps also by hydrophobic bonding [250]. The stabilizing effect of van der Waals forces would appear to be significant, since smaller cations such as aniline and benzocaine were found not to show such a strong interaction with PHEMA as chlorhexidine, though similar interactions

to that between PHEMA and chlorhexidine were envisaged with other organic cations [250]. Further studies indicated that the mechanism of interaction between chlorhexidine digluconate and PHEMA was consistent with an ion-ion interaction, and that the ionic nature of PHEMA was due to carboxylic acid sites which were thought to be generated in situ during polymerization [251].

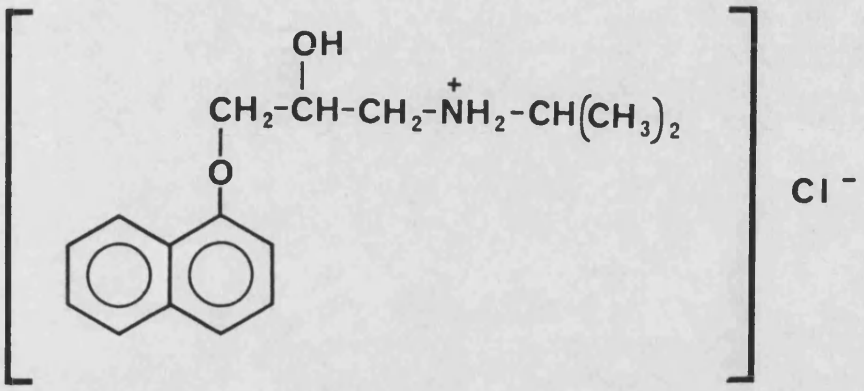
The effect of introducing methacrylic acid residues (carboxylic acid sites) into PHEMA on the diffusion of sodium chloride through crosslinked PHEMA films has been investigated [252]. The presence of methacrylic acid was reported to slow down the transport of sodium chloride through crosslinked PHEMA films primarily due to an exclusion effect on the chloride coion, the decrease in permeability being related to the methacrylic acid content in the copolymer. However it was not clear from the data presented at what pH the effect was examined or to what extent the carboxylic acid residues were ionised. The effect of pH on the transport of sodium chloride through the methacrylic acid/PHEMA copolymer was investigated, however the data presented did not clearly indicate whether an increase in pH resulted in an increase or a decrease in the diffusion of sodium chloride. The effect of methacrylic acid content on the transport of magnesium sulphate was also examined, however in this case no effect was reported. In this case, the effect was considered due to the larger size of the hydrated magnesium and sulphate ions whose transport is considered to be primarily controlled by the mechanical barrier imposed by the polymer network rather than coulomb interactions.

Chapter 2

Preparation and Characterization of Materials

2.1 Materials

Propranolol hydrochloride B.P. was received as a gift from Forum Chemicals Ltd., Redhill, U.K. Batch No. 3327. The chemical structure of propranolol HCl (molecular weight 295.8) is shown below :



2-Hydroxyethyl methacrylate (HEMA), stated purity > 97%. The monomer was supplied by Fluka Chemicals, through Fluorochem Ltd, Glossop, U.K. Batch Nos. 261062 286, 265451 686, 274343 1187.

Ethylene glycol dimethacrylate (EGDMA), stated purity 96%. The monomer was supplied by Fluka Chemicals, through Fluorochem Ltd, Glossop, U.K. Batch No. 200229 286.

Ammonium persulphate, electrophoresis grade, supplied by FSA Laboratories Supplies, Loughborough, U.K.

Diazald, stated purity 99%, supplied by Aldrich Chemical Co. Ltd., Dorset, U.K.

All other chemicals and solvents used were of 'analytical reagent' purity unless otherwise stated. Solvents used in HPLC analysis were all of HPLC grade. The preparation of all buffers used in this work is described in appendix 2.

2.2 Methods

2.2.1 Assay of Propranolol HCl by U.V. Spectrophotometry

A series of different concentrations of propranolol hydrochloride solutions were prepared, and a scan performed on a double beam spectrophotometer (550S, Perkin Elmer Ltd., Slough, U.K.), using 1 cm path length quartz cuvettes. The solutions were scanned to determine a wavelength suitable for u.v. assay and to examine the effect of pH on the u.v. absorption of propranolol HCl. The solutions were scanned between the wavelengths 190 and 400 nm. The solutions examined were as follows :

i) 9.67×10^{-5} M Propranolol HCl in distilled water

ii) 9.67×10^{-5} M Propranolol HCl in pH 3.03 buffer

iii) 9.67×10^{-5} M Propranolol HCl in pH 4.48 buffer

iv) 9.67×10^{-5} M Propranolol HCl in pH 10.05 buffer

Details of the buffers used are given in appendix 2.

2.2.2 Assay of Propranolol HCl by High Performance Liquid Chromatography (HPLC)

The u.v. absorption at 288 nm of a solution of propranolol HCl was found to be unaffected when drug degradation was known to have occurred (section 2.3.1), thus another assay method capable of discerning the degradation products of propranolol HCl was required. A modified HPLC assay [127] was used in order to quantify the degradation of propranolol HCl. The HPLC column used was a 15 cm length, 0.5 cm internal diameter stainless steel column, packed with octadecylsilane (ODS) 5 µm reverse phase packing material (Hypersil, Shandon Southern Products, Runcorn, U.K.). The column was maintained at a constant temperature of 37°C. The mobile phase used was as

follows :

Tetrabutylammonium hydroxide (40%)	25 ml
Phosphoric acid	8 ml
Methanol	450 ml
Distilled water	to 1000 ml

The mobile phase was degassed with helium at room temperature for approximately fifteen minutes, and the pH adjusted to 2.5 using phosphoric acid. A flow rate of 1.2 ml min^{-1} was maintained through the column. Two systems were used depending on the volume of the sample for assay. Samples produced as described in section 4.2.1.3 required the use of an automated system capable of assaying 0.2 ml samples. The system for general assay was composed of a sample injection valve (Rheodyne Inc., Cotati, U.S.A.) connected to a 25 µl injection loop. A constant flow pump (Constametric III G, LDC/Milton Roy, Staffordshire, U.K.) was used to drive the mobile phase through the column and through a u.v. detector (Spectromonitor III, LDC/Milton Roy, Staffordshire, U.K.). The u.v. detector was set to a wavelength of 288 nm, corresponding to one of the principal absorption wavelengths of propranolol hydrochloride (section 2.3.1). The data was obtained as a continuous absorbance plot on a chart recorder.

The automated system used for small sample volumes was composed of an autosampler (SP8110 autosampler, Spectra-Physics Ltd., St. Albans, U.K.) feeding samples to a high performance liquid chromatograph (SP8100 liquid chromatograph, Spectra-Physics Ltd., St. Albans, U.K.). The presence of propranolol HCl or its degradation products was quantified using a u.v. detector (SP8400 u.v. detector, Spectra-Physics Ltd., St. Albans, U.K.) connected to an integrator

(SP4200 computing integrator, Spectra-Physics Ltd., St. Albans, U.K.)

The actual quantification of propranolol HCl samples was by means of the external standard technique, where unknown samples are quantified by direct reference to known concentrations of propranolol HCl.

2.2.3 Stability of Propranolol HCl

Some factors affecting the stability of propranolol HCl have been reported [253]. For example it is known that propranolol HCl is affected by light: in aqueous solution it decomposes through oxidation of the isopropylamine side chain. This chemical change is accompanied by a reduction in the pH and discolouration of the solution. Solutions are most stable at pH 3 and decompose rapidly when alkaline.

The effect of pH on the aqueous stability of propranolol HCl was examined in the present study. A series of solutions containing propranolol HCl buffered at different pH's were prepared and stored in the dark under conditions of controlled temperature. One formulation was stored in the light and at room temperature (table 2.1).

The solutions were assayed periodically by HPLC (section 2.2.2) to detect the amount of propranolol HCl remaining. The solution of propranolol HCl in pH 10.03 buffer was designed to accelerate degradation in order to validate the HPLC assay (section 2.3.2).

Table 2.1 Formulation of solutions used in stability studies.

Solution	Storage Conditions
0.179 mM propranolol HCl in distilled water	Dark-25°C
0.179 mM propranolol HCl in pH 3.03 buffer*	Dark-25°C
0.179 mM propranolol HCl in pH 4.48 buffer*	Dark-25°C
0.179 mM propranolol HCl in pH 10.03 buffer*	Light-room temperature

*(Appendix 2)

2.2.4 Preparation of Poly(2-hydroxyethyl Methacrylate) (PHEMA)

Analytical grade monomers were used as received from suppliers without further purification. One reason for this was that simple purification techniques such as distillation are reported to be not entirely satisfactory since the rate of polymerization and trans-esterification reaction increase strongly with temperature [254,255]. The monomers may be purified by a complex procedure such as that involving extraction with hexane, distillation under vacuum and treatment with alumina, however even after this purification, low levels of impurity still remain [220,229]. The monomers were stored prior to use at 4°C in the dark in order to minimise any such degradation. In order to reduce variation within experiments, the same batch of polymer was used for any one set of experiments. It is known that the concentration of monomers used, together with the degree of crosslinking will influence polymerization and thereby alter the properties of the resulting gels [211,212,213]. The composition of polymers will be described by the terms %T and %C, where T denotes the total percentage of all monomers (v/v) in the final polymer formula and C denotes the percentage of crosslinker (v/v) in the monomer fraction. All polymers used in this work had a crosslinker content in the range 0 to 7%C (which corresponds to approximately 0 to 4.6 mole % crosslinking), all with a total monomer concentration of 62.5%T unless otherwise stated. A concentration of total monomer greater than 60% was chosen to ensure that homogeneous PHEMA was formed (section 1.6.1).

2.2.4.1 Preparation of Crosslinked PHEMA by Gamma Irradiation

The use of radiation polymerized, rather than chemically polymerized gels has been favoured by some workers [210,211] due to reported differences in the swelling properties of PHEMA between the two methods and also due to the fact that initiators such as ammonium persulphate are not required for irradiation polymerization since free radicals are produced by gamma rays.

The gamma source used in the experiments reported here was Caesium-137. The gamma dose rate measured was 5.5 J kg^{-1} min^{-1} [127]. A 1%C, 62.5%T polymer solution was prepared as described below in section 2.2.4.2 and 2 ml aliquots were filled into separate moulds. The moulds used for irradiation polymerization consisted of glass tubes having a diameter of 2 cm and a length of 4 cm. The bottom end was sealed using a plastic sealing film (Nesco film, FSA, Loughborough), and the top end sealed with a glass microscope cover slip smeared with vacuum grease (Apiezon N, Apiezon Products Ltd., London, U.K.). The moulds were sealed under nitrogen. and were subsequently exposed to the gamma source at ambient temperatures for 18 hours. This corresponded to a total irradiation dose of 594 krad.

2.2.4.2 Preparation of Crosslinked PHEMA by Chemical Initiation

The use of chemically polymerized gels has been reported by many workers [210,222,242,256], this method has been adopted for all the PHEMA films used in this work. Films were produced by polymerizing in an appropriate mould, as described below.

Each mould consisted of two thin glass plates having dimensions: 0.2 cm thickness, 22 cm length and 11 cm width, as shown in figure 2.1. Three aluminium strips were fixed onto the surface of one plate

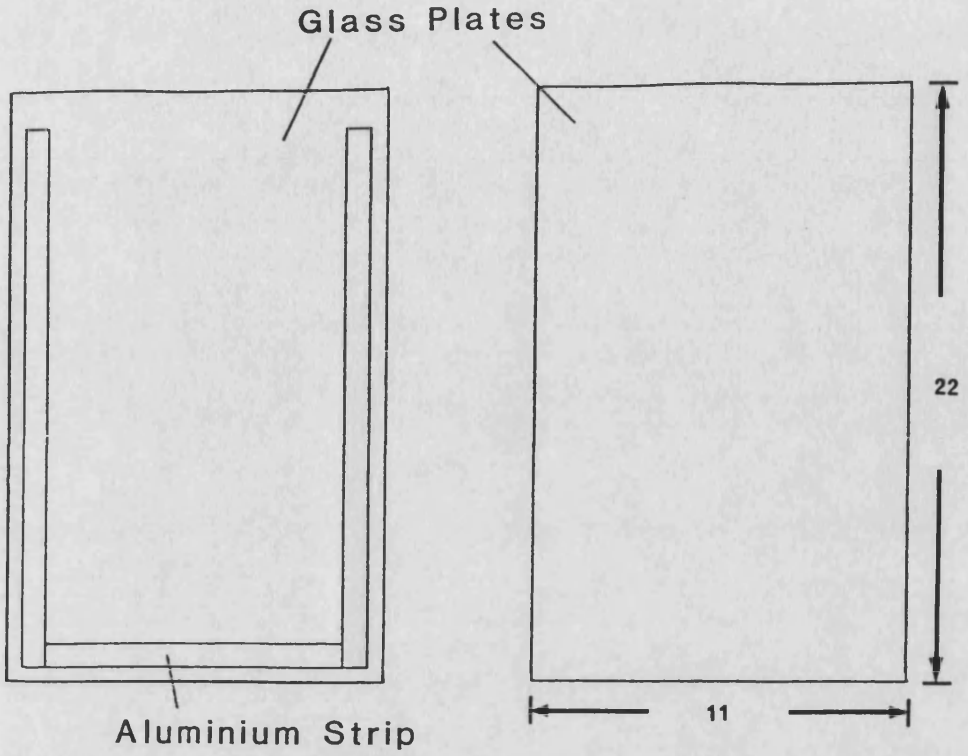


Figure 2.1. Mould used for the chemically initiated polymerization of PHEMA. The two halves of a mould prior to assembly are shown above, and a side view of the assembled mould is shown below.

around the perimeter of three of the plates edges using a silicone rubber adhesive (Silicone Rubber Compound, RS Components Ltd., Wetherby, U.K.) so that a gap was left between one of the short sides of the glass plate. The thickness of the aluminium strips determined the polymer film thickness. The mould was prepared for polymer film casting by sealing both plates together, spaced by the aluminium strips, using hydrogenated vegetable oil (Emvelop, Edward Mendell Co. Inc., New York, U.S.A.). This was achieved by heating both the moulds and the hydrogenated vegetable oil in an oven at 80°C prior to assembly, then carefully applying a layer of molten hydrogenated vegetable oil using a heated Pasteur pipette around the three edges of the mould having aluminium strips attached to them. The plates were then clamped together and allowed to cool, providing a water tight seal with one of the shorter edges of the plates being open to allow insertion of the polymerizing solution.

An example of a typical polymer formulation for 1%C, 62.5%T PHEMA was as follows :

EGDMA	0.2 ml
HEMA	19.8 ml
Distilled water	8.0 ml
4% ammonium persulphate	4.0 ml

The HEMA monomer is added to the distilled water, the solution being stirred rapidly using a magnetic stirrer, the EGDMA was then added slowly using a calibrated automated pipette (P200, Gilson, Anachem, Luton, U.K.). The mixture was then degassed for 12 minutes, using helium. The polymerization initiator, ammonium persulphate, was then added as a freshly prepared aqueous solution, to give a final concentration of 0.5%, and the solution degassed for a further 3

minutes. The mixture was then pipetted into the moulds using a Pasteur pipette. The moulds were partially immersed (up to the polymerizing solution level) in a water bath, which was then switched on and gradually allowed to heat up to 55°C so that the seal between the plates remained intact. Nitrogen was passed into a glass cover surrounding the plates at a slow, near-constant rate, so as to prevent contact of air with the polymerizing solution. The polymerization was allowed to proceed for 18 hours, after which the glass plates were separated and the polymerized PHEMA film peeled away. The plates were cleaned in a three stage process : firstly using a surfactant solution, then acetone and finally distilled water.

2.2.5 Swelling Rate of PHEMA in Solution

The swelling characteristics of PHEMA gels polymerized chemically were examined gravimetrically. The swelling of 0.83%C, 60%T PHEMA immersed in both water and pH 4.48 buffer (appendix 2) was investigated.

After polymerization was complete and the polymer removed from its mould (section 2.2.4.2), 4 cm diameter discs were immediately cut, blotted using absorbent paper to remove excess surface liquid, weighed and placed in separate beakers containing 40 cm^3 of either buffer or water. The beakers were immersed in a water bath maintained at 25°C. The gel discs were removed at specified time intervals, blotted to remove superficial liquid, weighed and then returned to fresh water or buffer as appropriate. The degree of swelling was estimated from the observed percentage change in weight which was

calculated as :

$$\% \text{ swelling} = 100 \times \frac{Wt-Wo}{Wo}$$

where :

Wt = weight of gel disc at a given immersion time

Wo = weight of gel disc prior to initial immersion

2.2.6 Equilibrium Water Content of PHEMA

The equilibrium water content of a range of crosslinked PHEMA films was examined (0-7%C), the water content of gels stored in both water and pH 4.48 buffer (appendix 2) was examined.

Polymer discs of 4 cm diameter were immersed in beakers containing either water or buffer maintained at 25°C in a water bath, the discs were weighed daily (section 2.2.5) until they had reached equilibrium swelling. The gel discs were weighed, then dried in a vacuum oven at 55°C over phosphorous pentoxide, and weighed periodically until they reached constant weight. The equilibrium water content was calculated as a percentage :

$$\% \text{ water content in PHEMA} = 100 \times \frac{Wh-Wd}{Wh}$$

where :

Wh = equilibrium weight of hydrated gel

Wd = weight of dried gel

2.2.7 Scanning Electron Microscopy of PHEMA

PHEMA gel structure was examined using a scanning electron microscope (JEOL JSM 35C, Japanese Electron Optics Ltd., Tokyo, Japan).

Discs of 1%C PHEMA were cut and rapidly frozen by immersion in liquid nitrogen. The gel discs were then removed using forceps and

fractured by striking with a hammer. The freeze fractured gels were then freeze dried overnight at -60°C (Edwards-Pearse Tissue Dryer EPD3, Edwards High Vacuum, Crawley, U.K.). Samples of the gel were mounted using a colloidal graphite adhesive on 2.6 cm diameter aluminium planchettes. A layer of gold was deposited onto the sample surface using a sputter coating technique (Edwards S15OB, Edwards High Vacuum, Crawley, U.K.). During coating a vacuum of 100 µm of mercury was drawn and a current of 20 mA at 1.4 kV was applied across the sample planchettes for six minutes. The gold coated samples were then imaged immediately in the scanning electron microscope using an electron beam energy of both firstly 5 kV and then 20 kV. The gold and graphite layers ensured that the specimens were fully earthed to prevent charge build up and overheating.

2.2.8 Diffusion of Propranolol HCl through PHEMA Films

The effect of PHEMA crosslinker content on the diffusion coefficient of propranolol HCl through crosslinked PHEMA films was investigated. The method used to determine diffusion coefficients was based on zero-order flux through a polymer membrane with defined boundary conditions (see also appendix 1). The diffusional transport of propranolol HCl across crosslinked PHEMA films was monitored from a reservoir (or donor) into a receptor compartment. The glass diffusion cell used is shown in figure 2.2. Each compartment of the cell had a capacity of approximately 220 ml. The cell was designed to satisfy the following criteria according to theoretical and experimental requirements :

i) Stirring must be adequate and constant in order to minimize boundary layers and to give reproducible analysis of diffusion

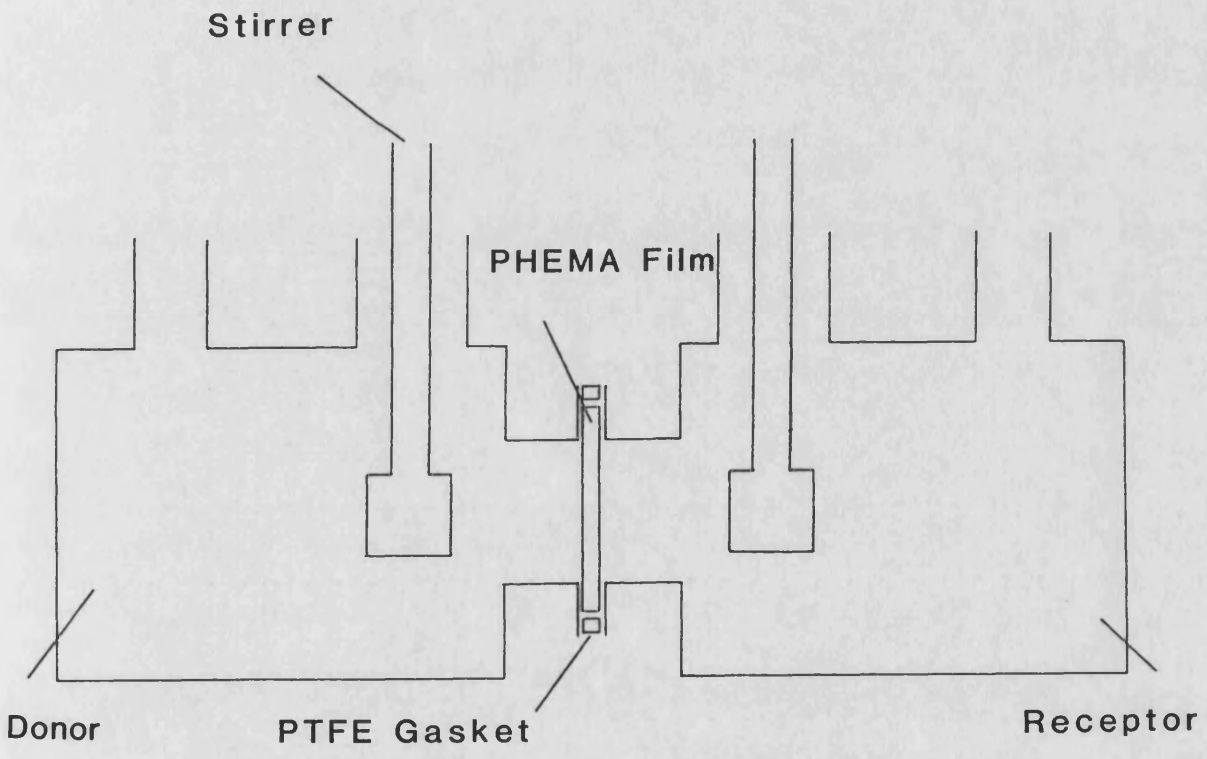


Figure 2.2. Schematic diagram of glass cell used to study the diffusion of propranolol HCl through crosslinked PHEMA films.

results.

ii) The cell interface must be designed to provide an efficient seal.

iii) The cell interface should not excessively strain the polymer film, which would result in distortion and could thus induce flaws in the film and produce erroneous or misleading results.

A range of crosslinked PHEMA films (0-7%C) of approximately 0.1 cm thickness were prepared as described above in section 2.2.4.2, and discs of 4 cm diameter were cut. The discs were stored in pH 4.48 buffer prior to use. The interfacial ground glass joints of the diffusion cell were smeared with a thin layer of vacuum grease (Apiezon N). An annular sheet of polytetrafluoroethylene (PTFE), with a thickness of 0.08 cm and internal diameter of 4 cm was used as a liquid-tight gasket. The PTFE gasket was placed at the interface of the donor cell, and a disc of polymer film placed in its centre. The diffusion cell was then clamped tightly and provided an exposed area of polymer film of 6.16 cm^2. Into the receptor compartment was placed 210 ml of pH 4.48 buffer, prepared as described in appendix 2, and into the donor was placed 200 ml of pH 4.48 buffer. The whole diffusion cell was mounted in a specially constructed perspex stand and immersed in a water bath maintained at 25°C. The compartments were stirred continuously using perspex stirrers driven by 12 volt motors at 330 rev min^{-1} (RS Components Ltd., Corby, U.K.), which were powered by a constant voltage power supply (E30/1 Farnell Instruments Ltd, Wetherby, U.K.).

The cell was allowed to equilibrate for 30 minutes, after which 20 ml of a stock propranolol HCl solution was added to the donor compartment to give a concentration of approximately 7 mM, timing was

started. After 15 seconds a 10 ml sample was withdrawn from the donor compartment using a pipette to give a measurement of the initial donor propranolol HCl concentration. This sample was analysed by u.v. spectrophotometry (section 2.2.1).

The diffusion coefficient was determined by monitoring the concentration of propranolol HCl in the receptor compartment. The receptor cell analysis, data collection and data manipulation were all automated. The receptor cell solution was pumped at a rate of 0.5 ml min^{-1} (Gilson Minipuls 2, Anachem, Luton, U.K.) into a flow-through u.v. detector (Gilson 116, Anachem, Luton, U.K.), and the outflow returned to the receptor cell. The u.v. detector, set to a wavelength of 288 nm corresponding to the wavelength of principal absorption for propranolol HCl (section 2.3.1), was connected to a microcomputer (BBC Master, Acorn Computers Ltd., Cambridge, U.K.), which collected the data at preprogrammed time intervals of one hour, converted them into concentration values, and stored all the data onto floppy disc at the end of each experiment. The data was then analysed using a statistics package (INSTAT, University of Reading, U.K.), which enabled diffusion parameters to be calculated from Barrer Plots, as described in appendix 1. A schematic diagram of the apparatus used is shown in figure 2.3.

The computer was calibrated by passing a number of solutions of known concentration through the u.v. detector. The programs used to calibrate the detector and collect data are given in appendix 3. A control experiment was performed in which no drug was added to the donor compartment to check for baseline drift. A second control experiment was also performed where a solution of approximately 4×10^{-5}M propranolol HCl was pumped through the u.v. detector. No

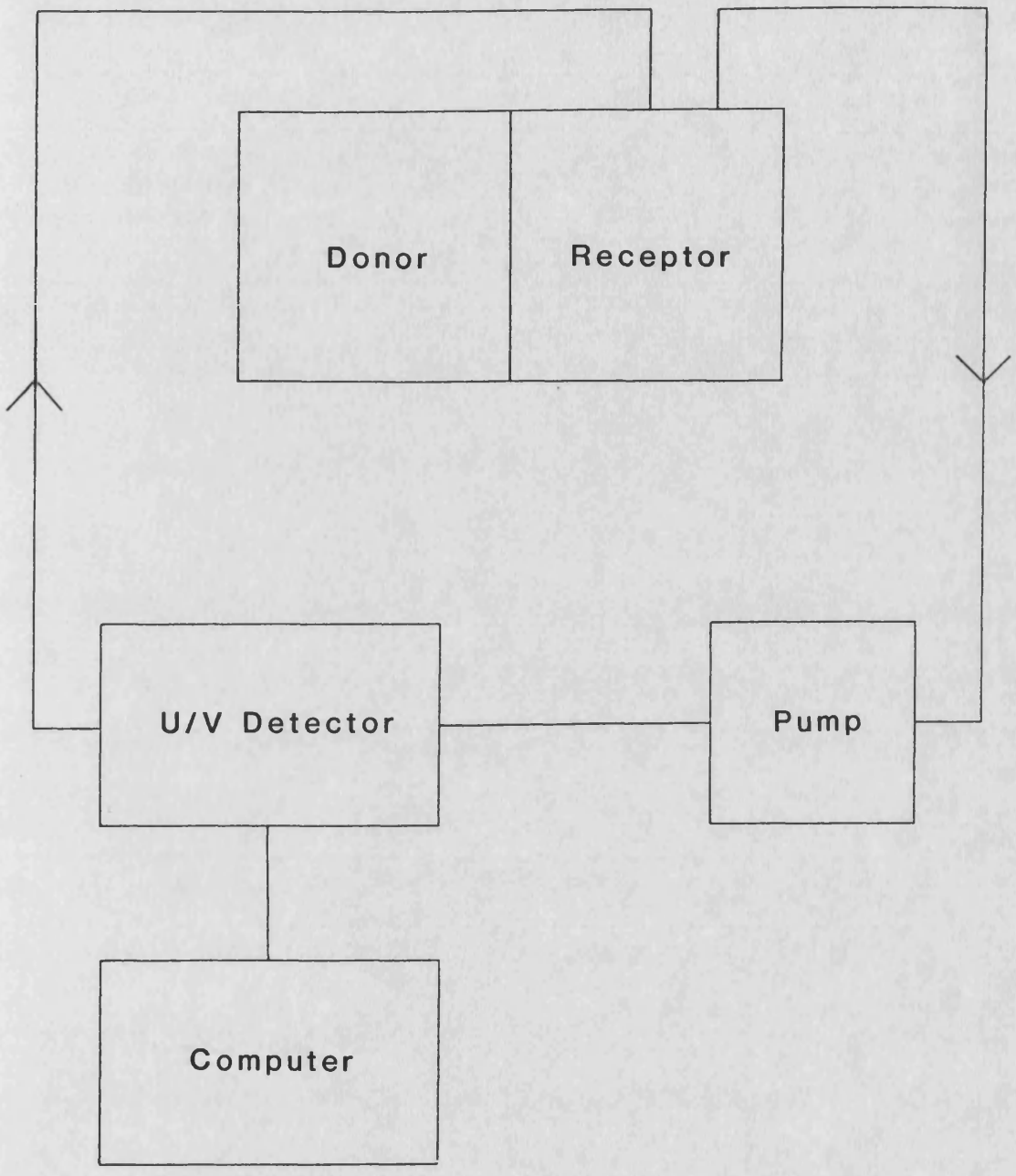


Figure 2.3. Schematic diagram of the apparatus used to measure the diffusion coefficient of propranolol HCl through crosslinked PHEMA films.

significant drift in either control was found over a period of five days under these conditions.

A series of crosslinked PHEMA films were investigated, three replicates being performed for any particular crosslinked hydrogel. At the end of each experiment the hydrogel film thickness was measured (section 2.3.4.2).

2.2.9 Uptake of Propranolol HCl by PHEMA

An interaction between the cationic preservative chlorhexidine and PHEMA has previously been shown in aqueous solution (as described in section 1.6.4) and it has been postulated that similar interactions may occur between PHEMA and other cationic drugs [250]. Thus in the experiments reported here the sorption isotherm of propranolol HCl in solution with PHEMA was investigated to see whether any similar interaction exists between PHEMA and propranolol HCl in solution.

Films of 1% crosslinked PHEMA, with a thickness of approximately 0.1 cm, were prepared as described in section 2.2.4.2, and discs of 1 cm diameter cut. The discs were then stored in pH 4.48 buffer (appendix 2) for approximately three weeks, the buffer being exchanged several times. The discs were then blotted with tissue to remove any excess surface liquid, and accurately weighed. The samples were then placed in a series of dry, 20 ml screw-top vials. A series of different concentrations of propranolol HCl in pH 4.48 buffer was prepared in the concentration range 0 to 3.4 mM. 10 ml of the appropriate propranolol HCl solution was then added to each vial. Five replicates were performed for each concentration. Samples of polymer in buffer without propranolol HCl, and samples of propranolol

HCl in buffer without polymer were also prepared in a similar way as controls. The vials were then inserted into racks which were placed in a thermostatted shaking water bath maintained at a temperature of 25°C, and shaken at a rate of 70 cycles min^{-1}. The racks ensured that the vials remained upright during the experiment. The samples were allowed to equilibrate for 21 days after which samples were taken from the vials, and following appropriate dilution were assayed by means of u.v. spectrophotometry (as described in section 2.2.1). The sorption isotherm was determined by calculating the equilibrium concentration of propranolol HCl, and the uptake of propranolol HCl by the PHEMA films.

2.2.10 Effect of Methylation of the Free Carboxyl Groups in PHEMA

The effect of methylating the free carboxyl groups in PHEMA by esterification using diazomethane was examined. The effect on the interaction of propranolol HCl with PHEMA was determined, together with the effect on the diffusion of propranolol HCl through crosslinked PHEMA films.

2.2.10.1 Methylation of PHEMA

Esterification of carboxyl groups in PHEMA was effected with diazomethane which is a selective methylating agent for acidic protons under the reaction conditions used [251,257,258], alcoholic protons being inert towards the methylating agent. The reaction may be expressed in the following manner :

$$CH_2N_2 + RCOOH \longrightarrow RCOOCH_3 + N_2$$

A range of crosslinked PHEMA films (0-7%C) of approximately 0.1 cm thickness and 4 cm diameter were prepared as described above

in section 2.2.4.2. Additionally 1 cm discs of 1%C were prepared for use in the uptake studies in section 2.2.10.3. The water in the polymer was exchanged with absolute ethanol by suspending the discs in 100 ml portions of absolute ethanol. The absolute ethanol was replaced daily over a period of two weeks.

Freshly distilled ethereal solutions of diazomethane were prepared [259]. Diazomethane production is a potentially explosive reaction, and therefore full safety precautions were necessary, including the use of scratch free glassware (Diazald Kit, supplied by Aldrich Chemical Co. Ltd., Dorset, U.K.). 50 ml of 96% ethanol was added to a solution of 10 g of potassium hydroxide in 15 ml of water, in a 200 ml distillation flask, mounted in a water bath maintained at 63°C. To the flask was connected a dropping funnel and an efficient condenser. The condenser was connected with a 500 ml round bottom receiving flask, which was cooled with an ice-salt mixture. A solution of 43 g of N-methyl-N-nitrosotoluene-4-sulphonamide (Diazald, supplied by Aldrich Chemical Co. Ltd., Dorset, U.K.) in 250 ml of ether was placed into the dropping funnel, which was adjusted so that the drop rate from it was approximately equal to the distillate drop rate. When the dropping funnel emptied, another fresh portion of approximately 25 ml of ether was added to the dropping funnel until the condensing distillate became colourless. The receiving flask containing ethereal diazomethane was then stored in an ice-salt mixture until ready for use.

Methylation of the polymer was achieved at room temperature, by adding 10 ml of the ethereal diazomethane to a series of conical flasks containing the polymer discs suspended in 100 ml of absolute ethanol. The polymer was allowed to stand for two hours, and the

suspending liquid poured into dilute acetic acid to destroy residual diazomethane. The process was repeated again with a fresh portion of absolute ethanol and another portion of ethereal diazomethane added to the flasks, and allowed to stand for a further two hours. The suspending liquid was then once again replenished with fresh absolute ethanol and ethereal diazomethane, and allowed to stand overnight. The absolute ethanol was then exchanged with water. This was achieved by suspending the discs in distilled water for a period of two weeks, replacing the water daily. The discs were then separated into three portions; one portion was retained for the diffusion studies in section 2.2.10.4, the discs being immersed in pH 4.48 buffer (appendix 2), which was replaced several times. The 1 cm, 1%C discs, also immersed in pH 4.48 buffer, were retained for use in the uptake studies in section 2.2.10.3. The final portion of 1%C discs were retained for assay to check for the presence of carboxyl groups (section 2.2.10.2).

2.2.10.2 Assay of PHEMA for Free Carboxyl Groups

An assay of the free carboxyl content in PHEMA by directly titrating the carboxyl groups with potassium hydroxide has been reported [251] and was adopted in these experiments.

Samples of 1%C PHEMA (prepared as described in section 2.2.4.2) and 1%C methylated PHEMA (prepared as described in section 2.2.10.1) stored in distilled water were used to assess the carboxyl content of PHEMA and also to assess the success of the methylation reaction described in section 2.2.10.1. The samples were firstly dried over phosphorous pentoxide in a vacuum oven at 60°C for 48 hours, with a fresh portion of phosphorous pentoxide used after 24 hours. The

polymer samples were then ground in an analytical mill (A10, Janke and Kunkel, supplied by Sartorius Instruments, Surrey, U.K.), and the powdered polymer stored in screw cap vials which were placed in a low humidity jar until ready for use. HEMA monomer was also analysed for carboxyl content.

The titration of carboxyl end groups in PHEMA required a standardised ethanolic solution of potassium hydroxide. A fresh solution of approximately 0.008 M potassium hydroxide in absolute ethanol was prepared daily and standardised against benzoic acid; a stream of nitrogen was bubbled through 20 ml of 0.008 M benzoic acid in absolute ethanol for five minutes to displace carbon dioxide. The solution was then titrated with the ethanolic potassium hydroxide, using two drops of phenolphthalein as indicator. 20 ml aliquots of absolute ethanol were also titrated to allow for solvent blank corrections.

The assay of the carboxyl groups was performed using approximately 1 g of either powdered PHEMA or 1 g of HEMA monomer suspended in 20 ml of absolute ethanol, in a glass stoppered two neck flask. Nitrogen was bubbled through the suspension for five minutes. The polymer suspension was then titrated with the ethanolic potassium hydroxide, using phenolphthalein as an indicator. The end point was considered to have been reached once a stable pink colour was obtained and which persisted for one hour, to allow diffusion of hydroxide ions into the polymer. The flask was frequently flushed with nitrogen during the titration.

The carboxyl content was determined in the form of millimoles of carboxyl groups per kilogram of polymer. Solvent blank corrections were taken into account in the calculations. Each assay was performed

in triplicate.

2.2.10.3 Uptake of Propranolol HCl by Methylated PHEMA

The uptake of propranolol HCl by PHEMA, expressed as a sorption isotherm, indicates an interaction between propranolol HCl and PHEMA in aqueous solution (section 2.3.9). A similar type of interaction has been shown between the cationic preservative chlorhexidine and PHEMA in aqueous solution, where the interaction was due to an ion-ion interaction between the free carboxyl groups in PHEMA and chlorhexidine [250,251]. In the present study the uptake of propranolol HCl by methylated PHEMA will be examined to see whether the interaction can be explained by a similar ion-ion mechanism.

Samples of 1%C methylated PHEMA, in the form of 1 cm discs were prepared (section 2.2.10.1). The uptake of propranolol HCl by the methylated polymer was determined according to the method described in section 2.2.9.

2.2.10.4 Diffusion of Propranolol HCl through Methylated PHEMA Films

The diffusion of propranolol HCl through films of crosslinked PHEMA films was found to be anomalous with respect to the effect which an alteration of the crosslinker content in PHEMA had on the diffusion coefficient of propranolol HCl through PHEMA (section 2.3.8). In the present study the effect of the carboxyl content in PHEMA on the diffusion coefficient of propranolol HCl through crosslinked PHEMA films was investigated, to examine whether this anomalous diffusion behaviour is a result of an ion-ion interaction between propranolol HCl and PHEMA.

4 cm Discs of methylated PHEMA films of approximately 0.1 cm thickness were prepared (section 2.2.10.1) in the crosslinker range 0 to 7%C. The diffusion coefficient of propranolol HCl through the films were determined according to the method described in section 2.2.8. Three replicates were performed for any particular crosslinked polymer.

2.3 Results and Discussion

2.3.1 Assay of Propranolol HCl by U.V. Spectrophotometry

Scans on solutions containing propranolol HCl in buffers ranging from pH 3 to 10 showed that the spectra were unaffected by pH in the region 190 to 400 nm. The spectra obtained (figure 2.4) showed absorption peaks characteristic of the naphthalene group found in propranolol HCl [260]. The peak at 288 nm was chosen for assay use since it was the principal absorption peak. A series of solutions of propranolol HCl in pH 4.48 buffer were prepared in the range 0 to 3.0×10^{-4}M from which a Beer-Lambert plot was constructed (figure 2.5). Linear regression analysis of the data produced the following statistics :

Number of data points	:	5
r^2	:	0.9999
Slope (S.D)	:	5700.2 (25.39)
Intercept (S.D)	:	6.986×10^{-3} (4.289×10^{-3})

The results showed a linear relationship and it was therefore considered that use of a u.v. assay was appropriate for analyses in the concentration range examined. The molar absorption coefficient, ε, was found to be 5700, corresponding to an E(1 per cent, 1cm) value of 192.7. These values are consistent with those that would be expected for a naphthalene group absorbing at a wavelength of 288 nm. The assay though satisfactory for quantifying concentrations of drug in solution was found not to be stability indicating. The absorbance value of a decomposing solution of propranolol HCl in pH 10.03 buffer (section 2.2.3) was found not to change with time, thus a u.v. assay alone would not be capable of distinguishing the presence of degradation products of propranolol HCl.

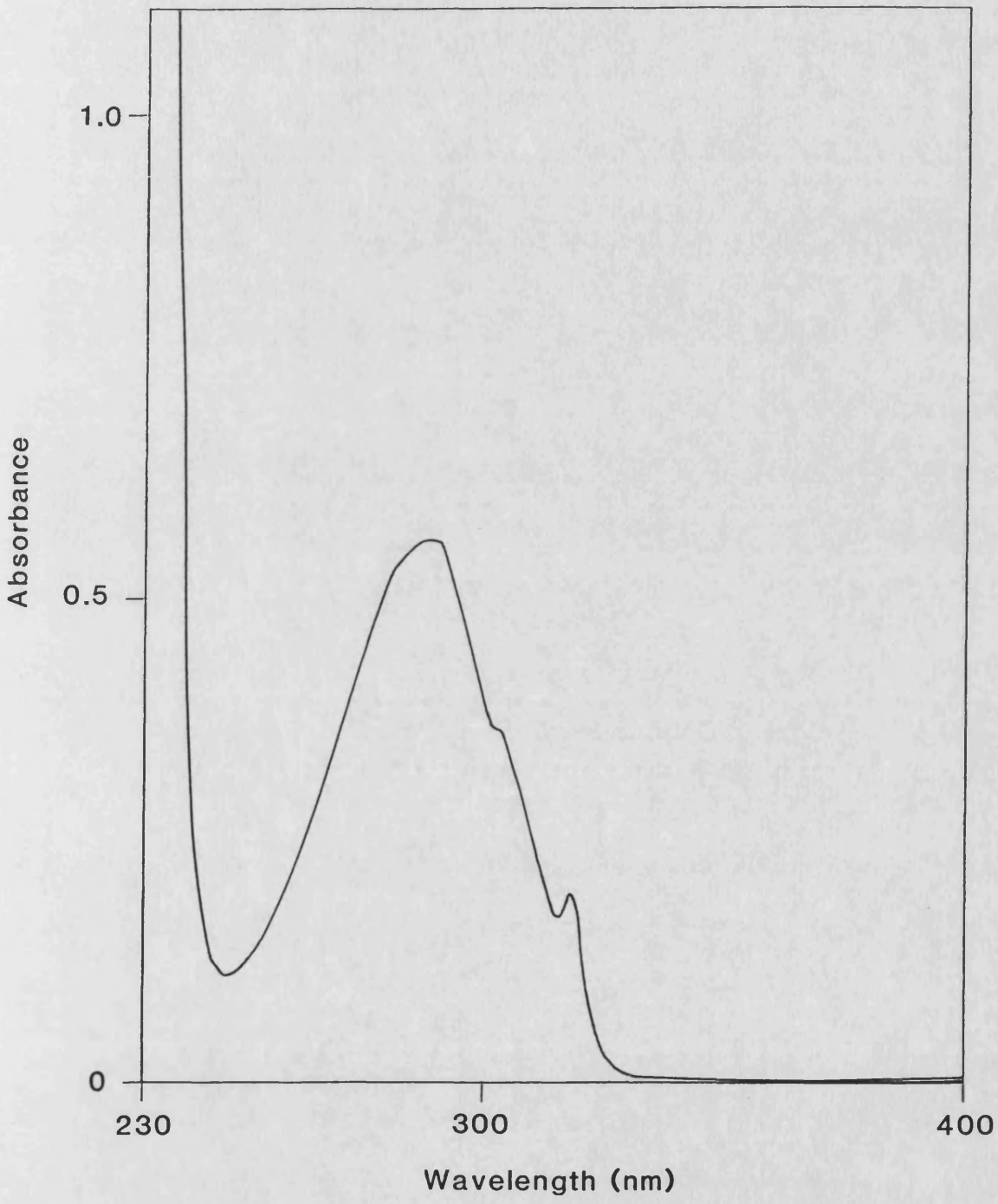


Figure 2.4. U.V. spectrum of propranolol HCl at a concentration of 9.67×10^{-5}M in pH 4.48 buffer.

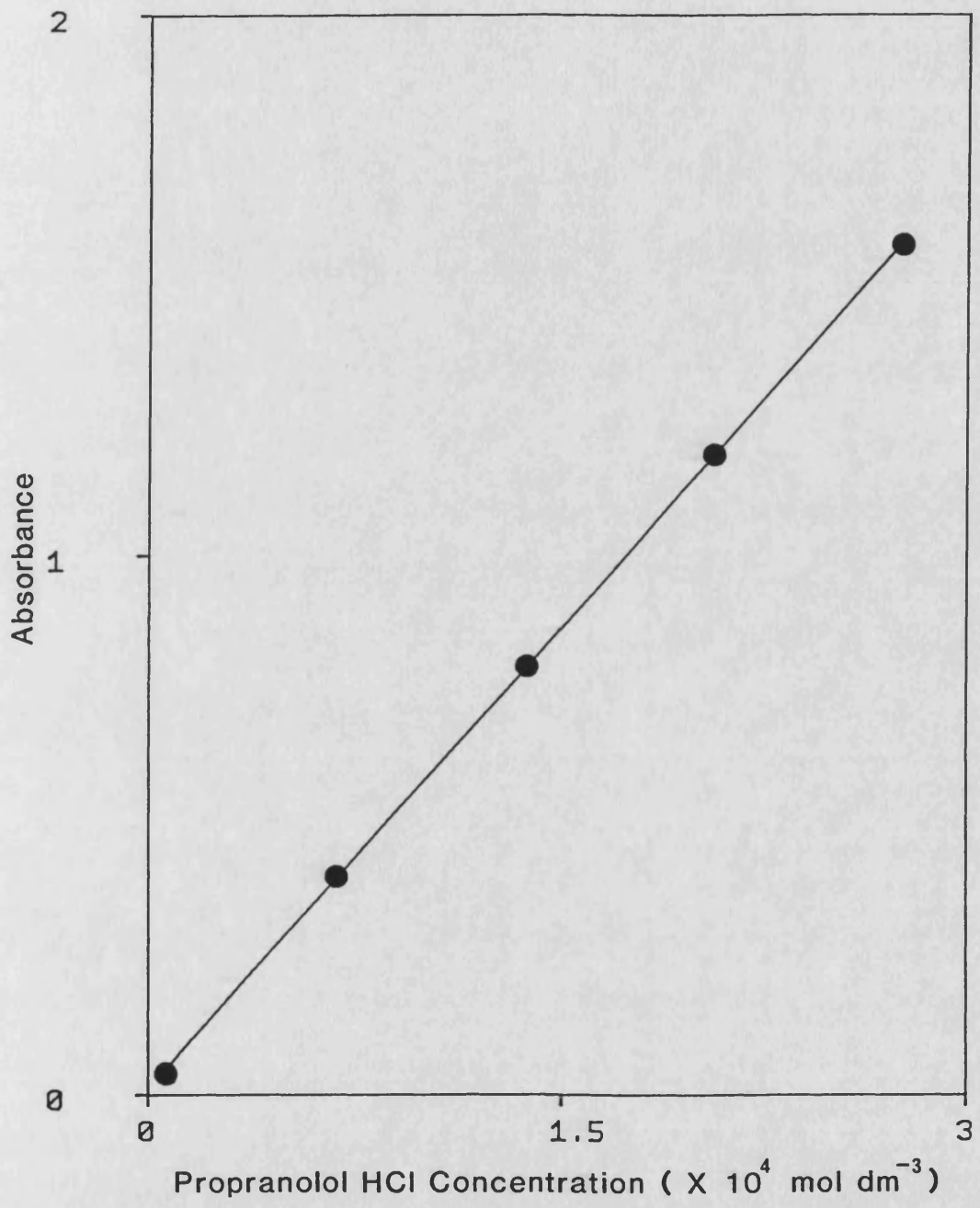


Figure 2.5. Beer-Lambert plot for propranolol HCl in pH 4.48 buffer at 288nm.

2.3.2 Assay of Propranolol HCl by High Performance Liquid Chromatography (HPLC)

An example of a HPLC chromatogram of propranolol HCl is shown in figure 2.6. The HPLC assay was shown to be stability indicating by analysis of data obtained by injecting decomposing samples of propranolol HCl in pH 10.03 buffer (section 2.2.3). The main propranolol peak, with a retention time of approximately 3.6 minutes, was found to decrease, accompanied by a decomposition product peak which increased as progressively older solutions were injected. Propranolol HCl in pH 4.48 buffer produced only one peak, with a retention time of approximately 3.6 minutes.

The relationship between peak area (and peak height) against concentration for propranolol HCl were found to be linear over the concentration range of 0 to 1.4 x 10^{-4}M. Sample solutions were prepared in pH 4.48 buffer (appendix 2) and were injected in duplicate to give a mean value for peak area (and height). A calibration curve is shown in figure 2.7 of mean peak area against concentration. The accompanying linear regression analysis data is shown below :

Number of data points	:	5
r^2	:	0.9997
Slope (S.D)	:	2.412 X 10^9 (2.391 X 10^7)
Intercept (S.D)	:	-4344.7 (1983.3)

The results show a linear relationship thus the HPLC assay using the external standard method was deemed satisfactory. The assay was also shown to be stability indicating.

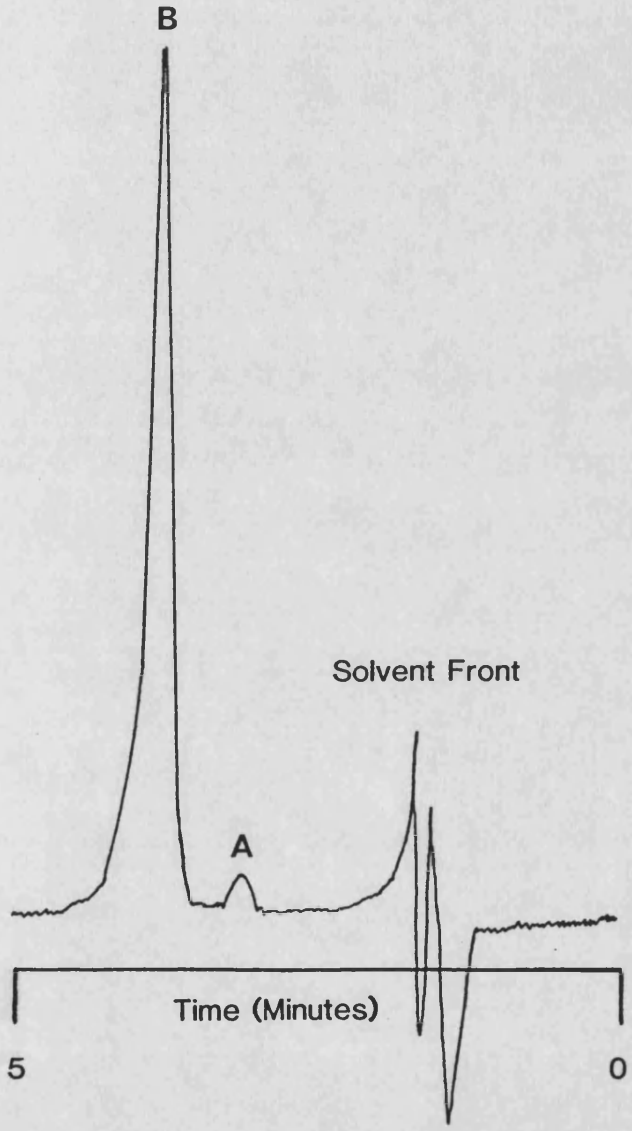


Figure 2.6. HPLC chromatogram of propranolol HCl (9.67×10^{-5}M) in pH 10.05 buffer showing a degradation product peak (A) and propranolol HCl peak (B).

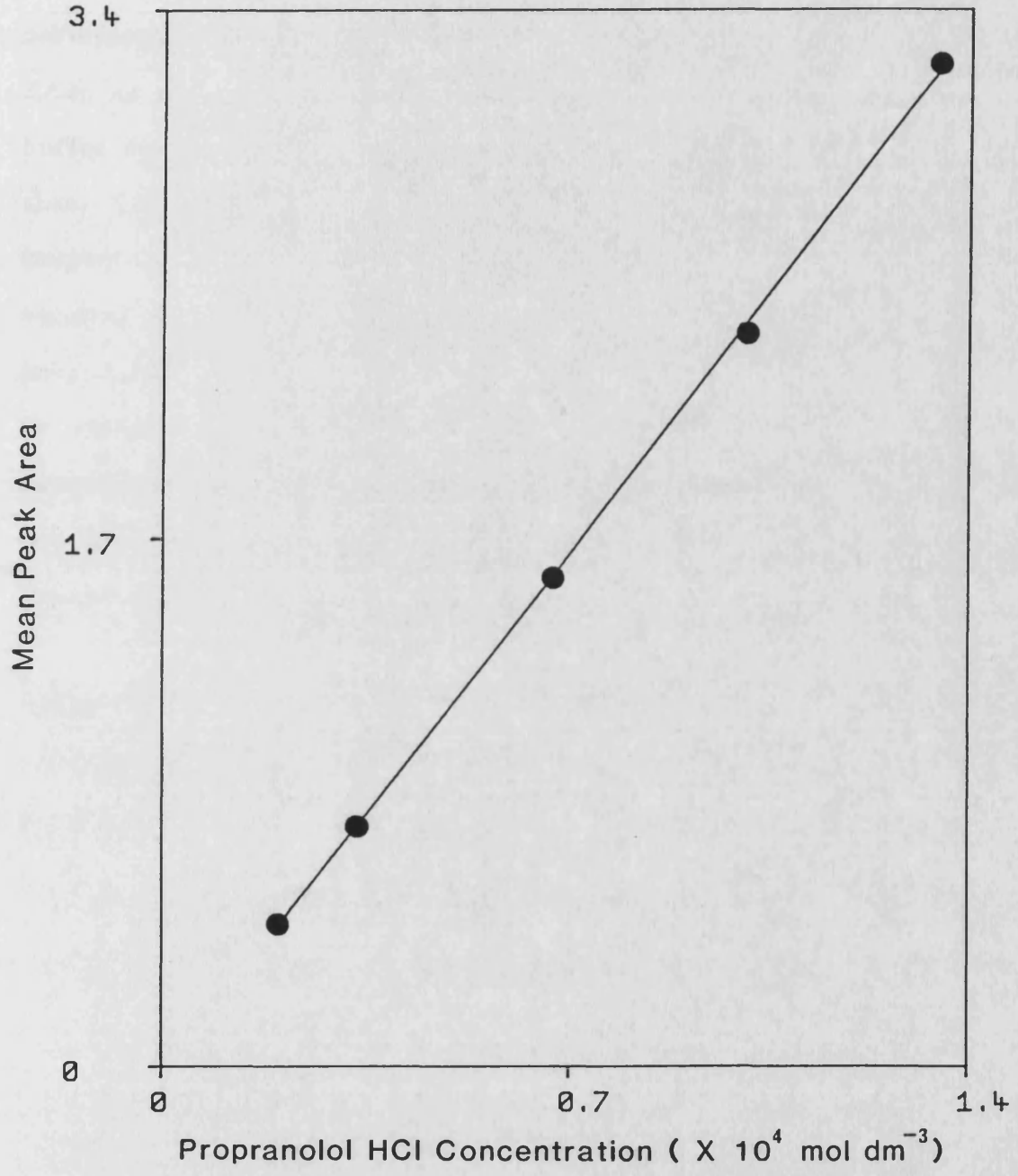


Figure 2.7. HPLC calibration plot for propranolol HCl.

2.3.3 Stability of Propranolol HCl

The samples prepared for stability studies were analysed periodically over a period of three months using HPLC (section 2.2.2). As expected the solution of propranolol HCl in pH 10.03 buffer degraded visibly, with the solution turning a light brown with time. The amount of propranolol HCl remaining in solution with respect to time was monitored for all four solutions. The pH of the aqueous solution was found to be 5.33 (appendix 2). The percentage of propranolol HCl remaining in solution with time and the effect of pH is shown in table 2.2. The results show that propranolol HCl decomposes rapidly at a pH of 10.05, but is stable for at least one month if stored at a pH below 5.33 and at a temperature of 25°C protected from light.

Table 2.2 The percentage of propranolol HCl remaining in solution with time when stored at various pH's. (See section 2.2.3 for storage conditions)

	pH			
Time	3.03	4.48	5.33	10.03
10 Days	100	100	100	88
23 Days	100	100	100	66
37 Days	100	100	100	50
3 Months	98	98	94	0

2.3.4 Preparation of Poly(2-Hydroxyethyl Methacrylate) PHEMA

2.3.4.1 Preparation of Crosslinked PHEMA by Gamma Irradiation

Successful polymerization could not be achieved using gamma irradiation. Polymerization was found to be incomplete after 12 hours, however after 18 hours the polymerization was found to be complete. The resultant polymer however was unsatisfactory. Macroscopic examination of the polymer showed that its shape was deformed and had an uneven surface, there were also air bubbles within the structure. The same problem has been previously encountered using the same radiation source [127]. The cause of the problem is probably related to a number of factors. Due to the nature of the source, samples are in very close proximity to the source which may result in uneven irradiation. In addition, due to the physical location of the source, it is not possible to exclude air from the polymerizing system. Another problem was that no temperature control could be effected during polymerization. In view of these problems and the nature of the radiation source it was decided to use chemically polymerized PHEMA in all subsequent work.

2.3.4.2 Preparation of Crosslinked PHEMA by Chemical Initiation

The method of solution polymerization produced homogeneous, transparent, ductile films. The films were free of any air bubbles and were easily removed from the glass moulds. The effect on the physical properties of increasing the crosslinker content was to decrease the polymers ductility. The films produced were homogeneous, however it was found that if higher water contents than about 42% were used, heterogeneous films were produced due to phase separation. These heterogeneous films were visibly different being white, opaque

and spongy in nature. The critical value of water that causes this turbidity is determined by amongst other things, the crosslinker content in the film [214].

The films were stored in distilled water in a fridge at 4°C, the distilled water being changed daily for 21 days. The purpose of this being to leach out any ammonium persulphate or unreacted monomers, and to allow the polymer to swell to its equilibrium volume [255]. The polymer was then cut into disc form and immersed in either buffer or water, depending on the intended experiment, and stored in the dark at 4°C until ready for use.

The hydrogel film thickness was measured by placing it between two glass plates of known thickness and measuring the total thickness with a precision digital micrometer (Mitutoyo, supplied by RS Components Ltd., Corby, U.K.), using the torque control so as not to compress the hydrogel. The films were found to be approximately 0.09 cm in thickness.

2.3.5 Swelling Rate of PHEMA in Solution

The results obtained for the swelling of PHEMA in water (figure 2.8) show that the polymer attained equilibrium after approximately 48 hours, the same type of profile has been obtained independently by other workers [209]. Some work has demonstrated anomalous swelling of chemically polymerized PHEMA [210] where the swelling exhibited a maximum followed by a minimum, the polymer not reaching equilibrium for about 14 days. This behaviour was ascribed to adsorption of initiator persulphate ions onto the polymer chains, however this effect was not seen in this work. The swelling in acetate buffer produced an unexpected profile (figure 2.8), an initial maximum was

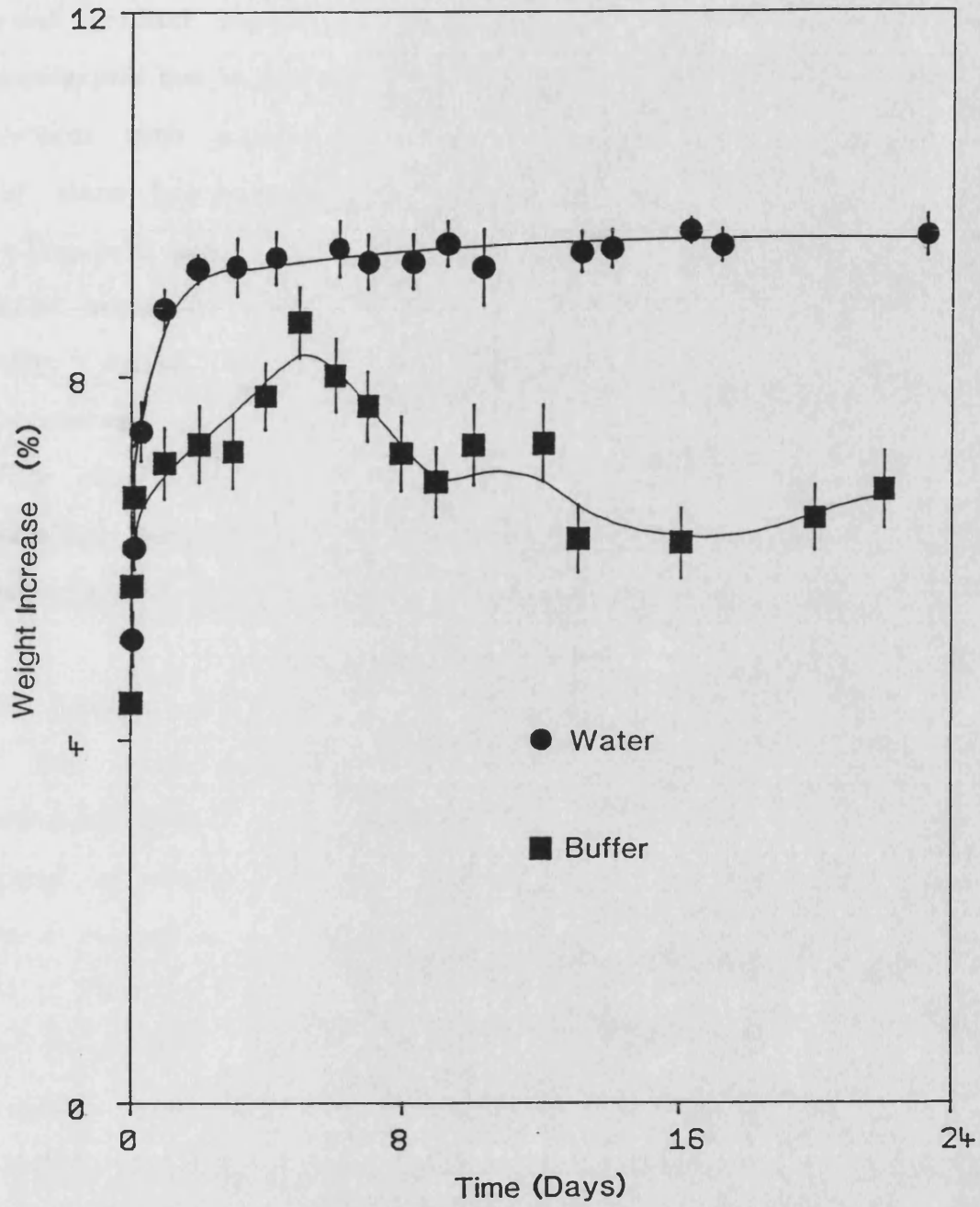


Figure 2.8. Percentage weight increase (with 95% confidence limits) versus time of PHEMA after polymerization when the polymer is immersed in :

a) distilled water.

b) pH 4.48 buffer.

found to be followed by a minimum which was in turn followed by a second gradual reswelling. This anomalous swelling behaviour was reproducible but is not easily explained. The swelling of PHEMA in solutions with other solutes including sodium acetate and acetic acid alone has been examined [210], each producing a different swelling behaviour attributed to the fact that with acetic acid, mainly unionised solute exists which can penetrate the polymer easily, unlike in the fully ionised sodium acetate. Factors determining solute effects on swelling include osmotic activity, solute size, and effects on polymer network bonding. Thus the anomalous behaviour seen in acetate buffer may be a complex combination of different effects on polymer swelling.

2.3.6 Equilibrium Water Content of PHEMA

The effect of crosslinker content on the equilibrium water content of PHEMA is shown in figure 2.9. The equilibrium water content of PHEMA stored in acetate buffer is slightly greater than that of polymer stored in water for all crosslinker contents. In the case of PHEMA stored in both water and buffer we see a relatively small decrease in equilibrium water content as crosslinker content is increased. This effect has been attributed to the network structure of PHEMA, in particular to the non-covalent secondary structure (section 1.6.1). The secondary structure, superimposed onto the covalent network is thought to control the swelling behaviour of the hydrogel. The equilibrium water content of PHEMA stored in water has been looked at by other workers, similar results being found [213,261]. Though increasing crosslinker content only decreases water content by a relatively small amount, the actual nature of water

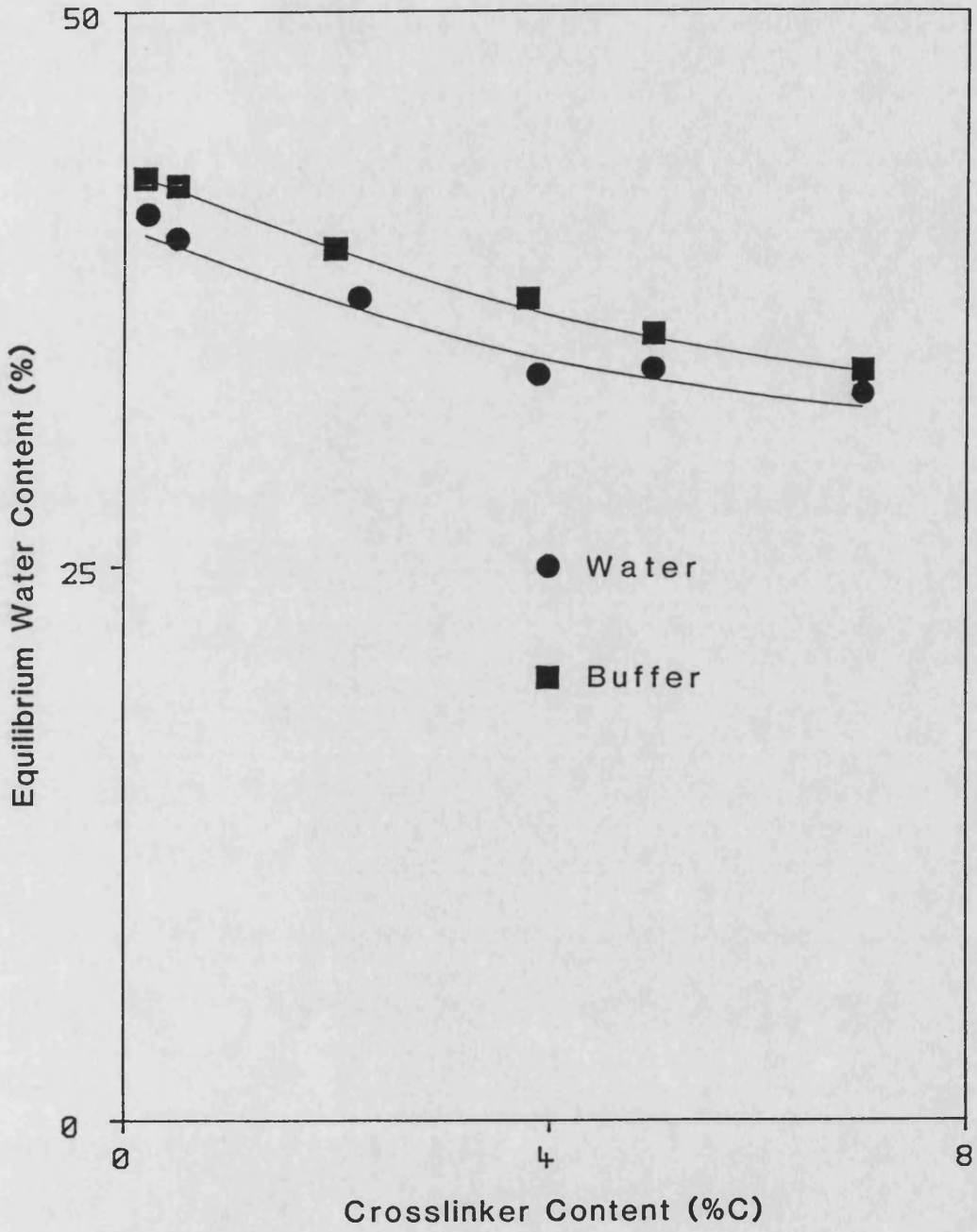


Figure 2.9. Effect of crosslinker content on the equilibrium water content of PHEMA when immersed in :

a) distilled water.

b) pH 4.48 buffer.

within the hydrogel is thought to change significantly, the percentage of bulk water decreasing, accompanied by an increase in the percentage of bound and interfacial water (section 1.6.1). These subtle changes can have a significant effect on the diffusion behaviour of solutes through PHEMA (section 1.6.3, section 2.3.8).

2.3.7 Scanning Electron Microscopy of PHEMA

Electron micrographs of PHEMA are shown in figure 2.10. The low magnification micrograph shows an exposed fracture surface of the hydrogel (figure 2.10a), at this magnification the polymer surface appears quite smooth. The maximum magnification that could be achieved was limited by the electron beam energy. The use of high energy beams on a localised area resulted in destruction of the PHEMA surface, seen visibly as blistering. The highest magnification that could be achieved was X5000 (figure 2.10b), with a beam energy of 5 kV. At this magnification no details of the polymers ultrastructure could be seen. Similar results have been found by other workers [262], however some ultrastructure was visible when the sample was frozen and observed at liquid nitrogen temperatures, allowing the sample to warm up slowly. This results in sublimation of the ice to reveal a fine structure, however the authors suspect the structure could be an artefact. Other SEM work on porous PHEMA has revealed an ultrastructure on the surface of PHEMA [212], but there were no details provided of preparation of the samples for microscopy.

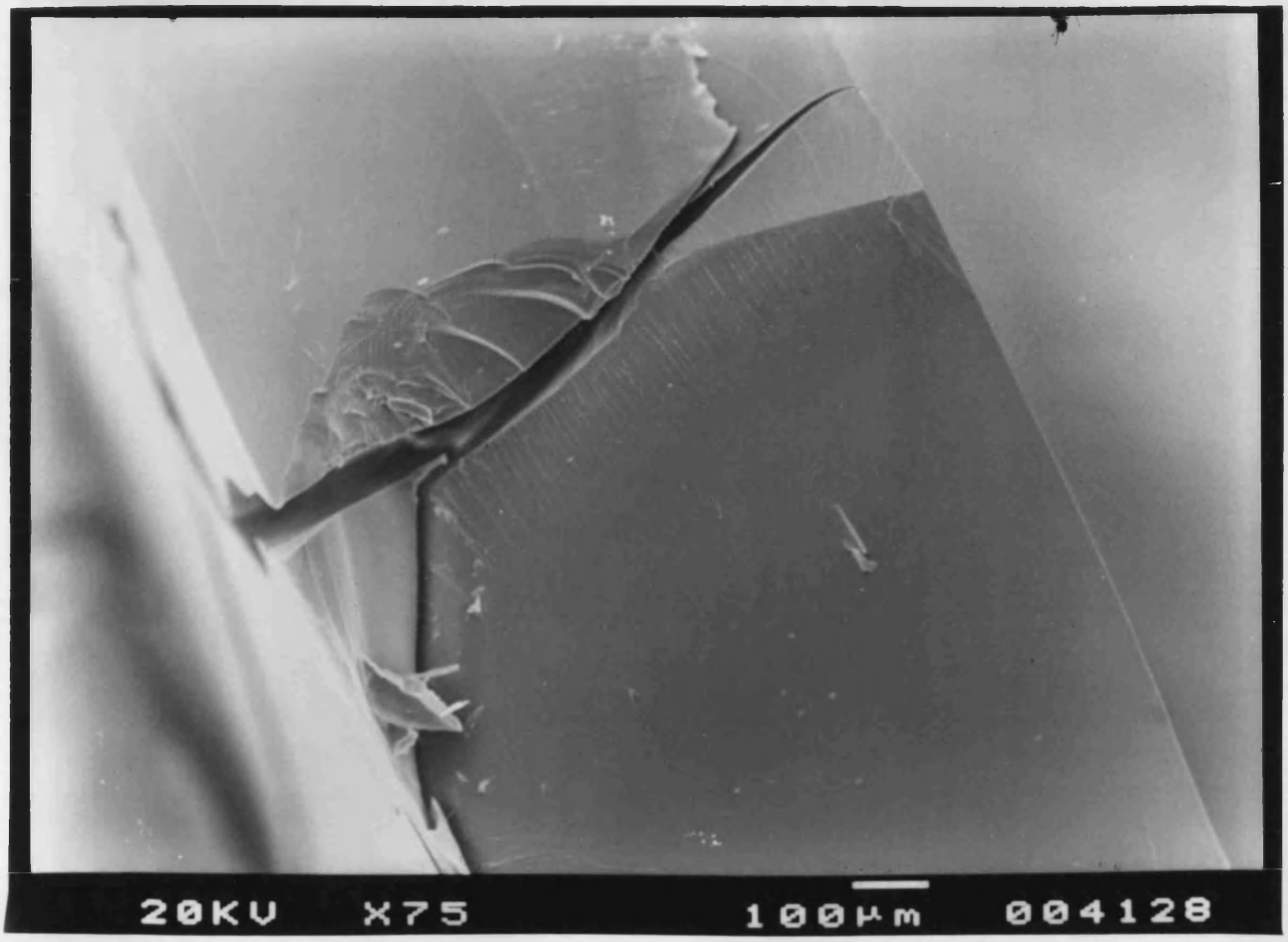


Figure 2.10a. Electron micrograph of 1% crosslinked PHEMA (X 75).

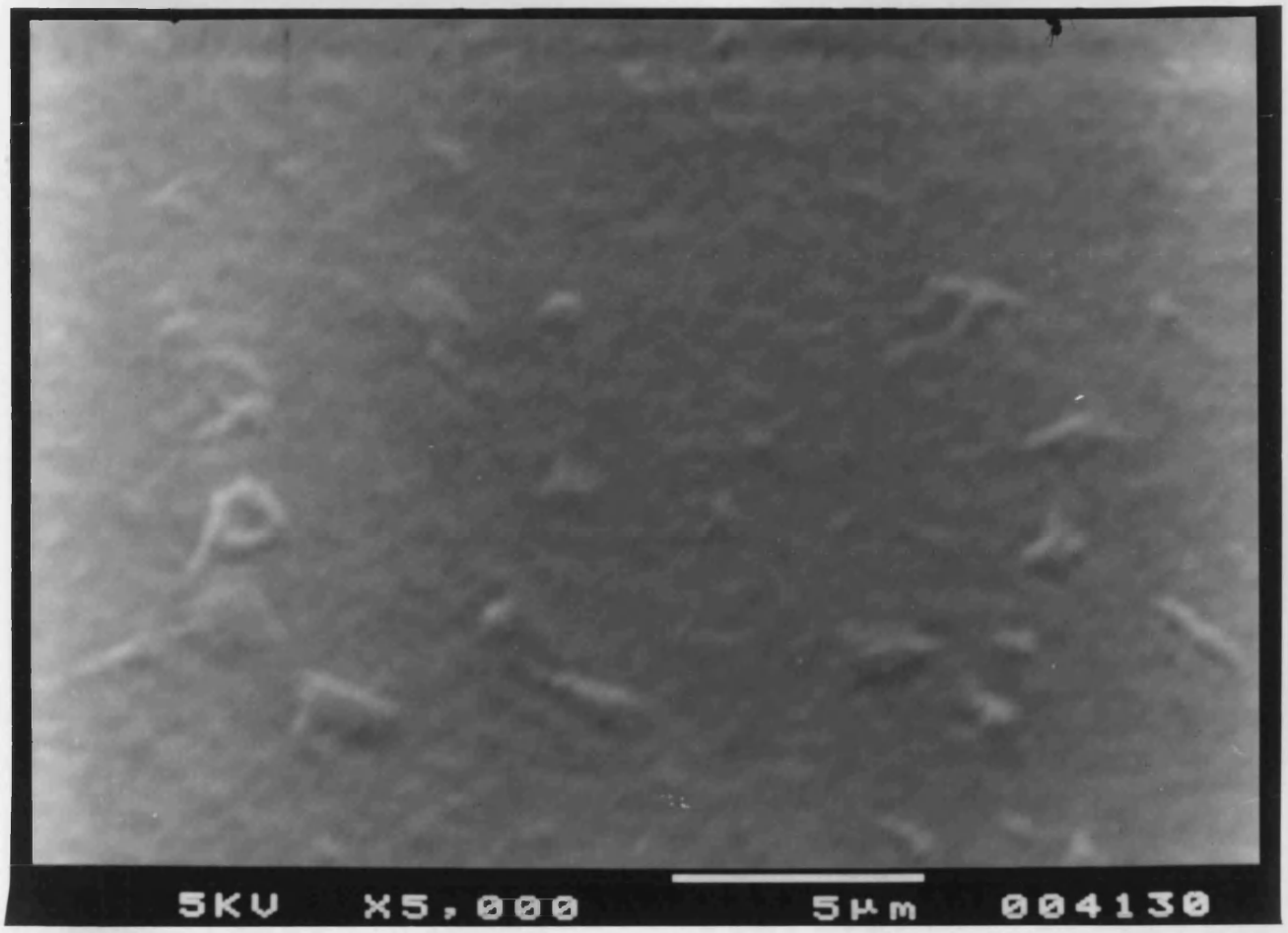


Figure 2.10b. Electron micrograph of 1% crosslinked PHEMA (X 5000).

2.3.8 Diffusion of Propranolol HCl through PHEMA Films

A typical Barrer Plot showing the concentration of propranolol HCl in the receptor compartment against time is shown in figure 2.11. The advantages of using an automated data collecting method can be seen in the large number of data points that can be collected, facilitating the analyses of data. The diffusion coefficients of propranolol HCl through the films of PHEMA were calculated from the lag times of the Barrer Plots (appendix 1), steady state not being assumed to have been reached until at least 2.7 times the initial lag period was achieved. Figure 2.12 shows the relationship found between the diffusion coefficient of propranolol HCl through PHEMA films and crosslinker content, each datum point representing the mean of three replicates. The results show that as crosslinker content is decreased, the diffusion coefficient increases rapidly, then tails off.

There are two main factors affecting the diffusion characteristics of solutes through PHEMA, namely the crosslinking density and the organisation of water within the gel [244]. There have been relatively few studies investigating the effect of crosslinking density on the diffusion coefficient of a particular solute [263], however those that have been carried out have shown a relationship which differs from that found with propranolol HCl [242,243,245,264]. Similar results are found at higher crosslinker contents, however the tailing at lower crosslinker contents was not found in any previous work, other workers having found that the diffusion coefficient tends to increase more rapidly as crosslinker content is decreased.

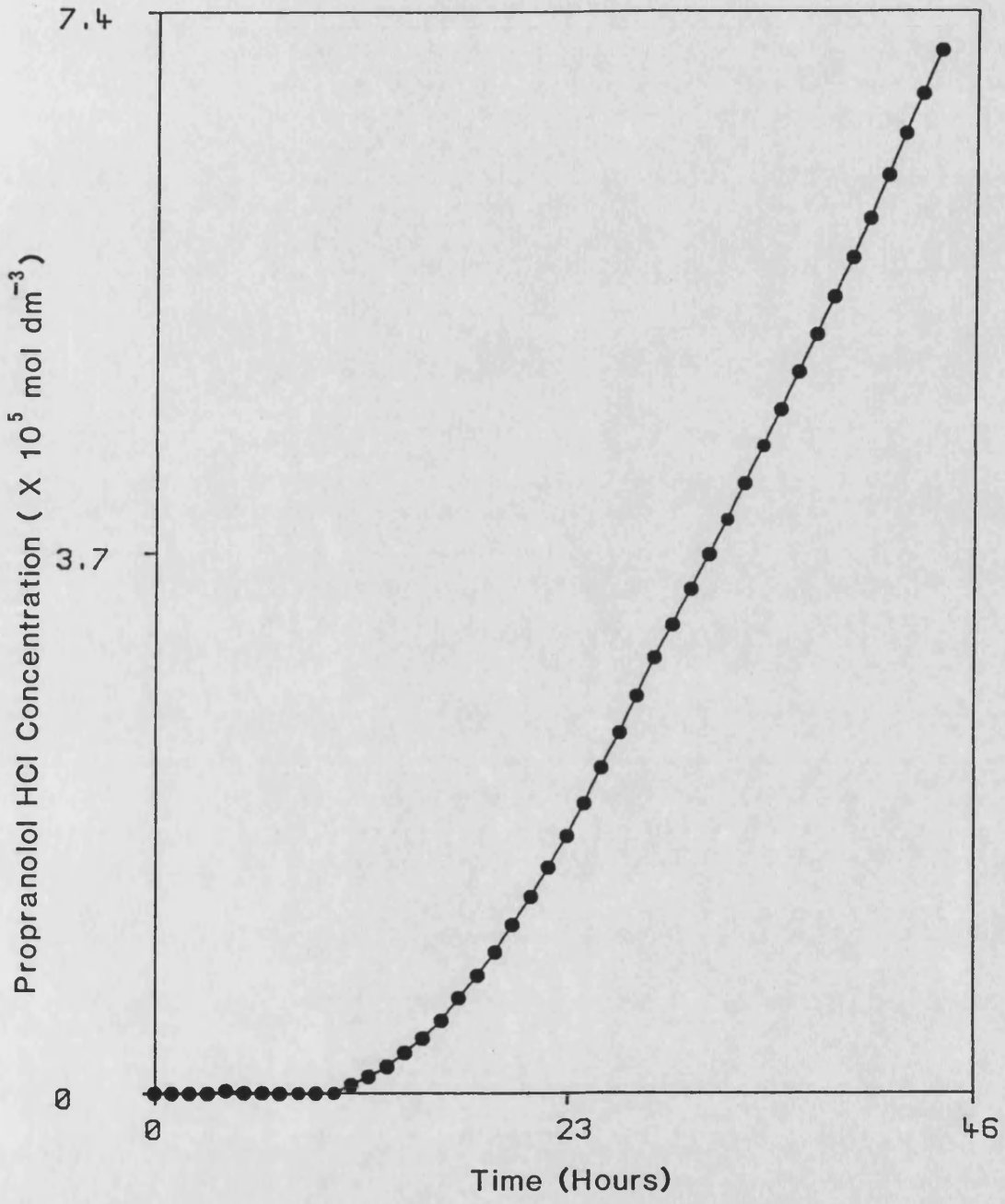


Figure 2.11. Barrer Plot for the diffusion of propranolol HCl through PHEMA, with the appearance of propranolol HCl in the receptor shown versus time.

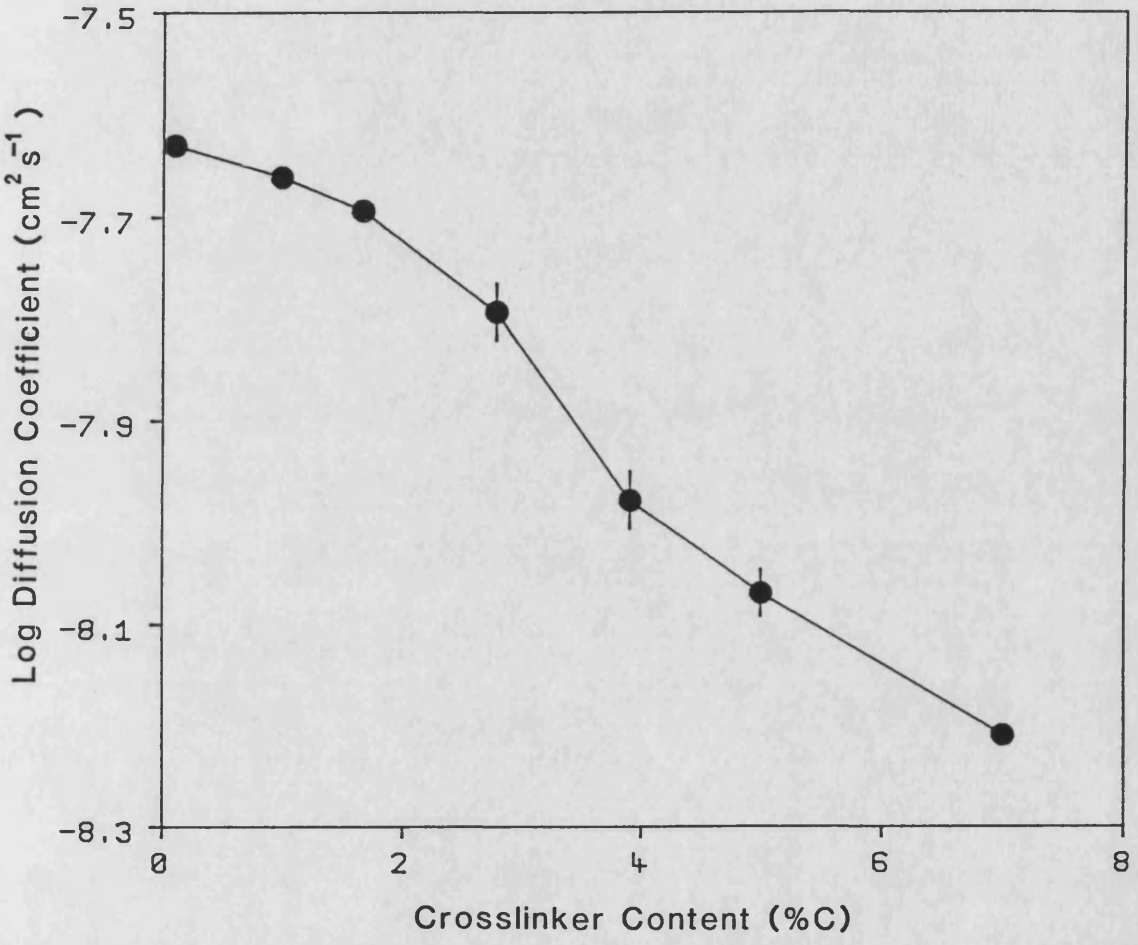


Figure 2.12. Effect of crosslinker content in PHEMA on the diffusion coefficient of propranolol HCl through crosslinked PHEMA films (with standard error bars shown).

The effect of crosslinker content on the diffusion coefficient in crosslinked PHEMA can be related to changes in the diffusion mechanism that occur (section 1.6.3). At low crosslinker contents a predominantly pore mechanism of diffusion exists as a result of the bulk water within the hydrogel. As the crosslinker content is increased a decrease in pore size is considered to occur along with changes in the water composition within the hydrogel, and solute diffusion is now predominantly the result of the so-called partition mechanism, where a solute is thought to be transported along the polymer backbone, via the bound and interfacial water fractions. These changes in mechanism can be seen in figure 2.12 with propranolol HCl, where at low crosslinker contents (about 3%C and less) a predominantly pore mechanism of diffusion probably exists, but as the crosslinker content is increased, diffusional transport becomes predominantly partition mechanism dominated. For the generalized condition it is considered that both mechanisms probably occur synchronously, with one of the two predominating in specific conditions.

It would be expected from previous work, and from theory, that the diffusion coefficient should increase more rapidly as the crosslinker content is decreased. It was thought that the cause of the anomaly found in the present study may result from an interaction between propranolol HCl and PHEMA in aqueous solution; this is considered further in section 2.3.9.

In support of the proposed change in mechanism of diffusion are the changes in partition coefficients obtained as the crosslinker content is increased. Partition coefficients were determined from the gradient of the steady state portion of the Barrer plots (see

appendix 1), using the diffusion coefficients obtained from lag times to give an estimate of the partition coefficient. The general trend found was a decrease in the value of the partition coefficients as crosslinker content was increased as shown in table 2.3. It should also be noted that for all crosslinker contents the partition coefficients are greater than one, which suggests that propranolol HCl may be interacting with the polymer.

The changes in equilibrium water content of PHEMA as crosslinker content is increased also supports the proposed changes in diffusion mechanism (section 2.3.6). The changes in the diffusion coefficient are consistent with an expected decrease in the proportion of Domain B water accompanied by an increase in the proportion of Domain A water as crosslinker content is increased and equilibrium water content decreased (section 1.6.1).

Table 2.3 Variation in the partition coefficient of propranolol HCl between PHEMA and bathing solution as crosslinker content is increased.

Crosslinker Content (%C)	Partition Coefficient (S.D.)
0.1	10.5 (0.3)
1.0	9.2 (0.6)
1.7	8.8 (0.4)
2.8	6.4 (1.3)
3.9	8.2 (1.5)
5.0	7.7 (1.4)
7.0	5.1 (0.1)

2.3.9 Uptake of Propranolol HCl by PHEMA

The sorption isotherm at 25°C of propranolol HCl in buffered solution with PHEMA is shown in figure 2.13. The control samples showed that there was no measurable loss of propranolol HCl to the glass vials, and that the PHEMA samples did not leach any substances detectable by u.v. assay.

The isotherm obtained was found to be similar to that obtained for chlorhexidine digluconate in aqueous solution with PHEMA [249], where an initial steep portion is found, which then decreased in steepness. This type of isotherm has been classified as a high affinity (H1) isotherm [246], and is associated with a surface adsorption process where a solute has a high affinity for adsorbent, however one would expect the isotherm to plateau as the polymer becomes saturated, though this was not found with either chlorhexidine digluconate, or propranolol HCl studied in this work, perhaps because the concentrations examined were too low.

It has been suggested that the interaction between chlorhexidine digluconate and PHEMA is due to an ion-ion interaction between carboxyl groups in PHEMA and the positively charged chlorhexidine ion (see section 1.6.4), the sorption isotherm perhaps being a composition of an initial portion representing a high affinity uptake, superimposed on which is an interaction with the polymer matrix [251]. This would perhaps explain why no plateau is found in the sorption isotherm.

It has been speculated that the type of interaction between chlorhexidine and PHEMA may be a feature of other positively charged ions besides chlorhexidine [250], thus the cause of the interaction between propranolol HCl and PHEMA in buffered solution may also be

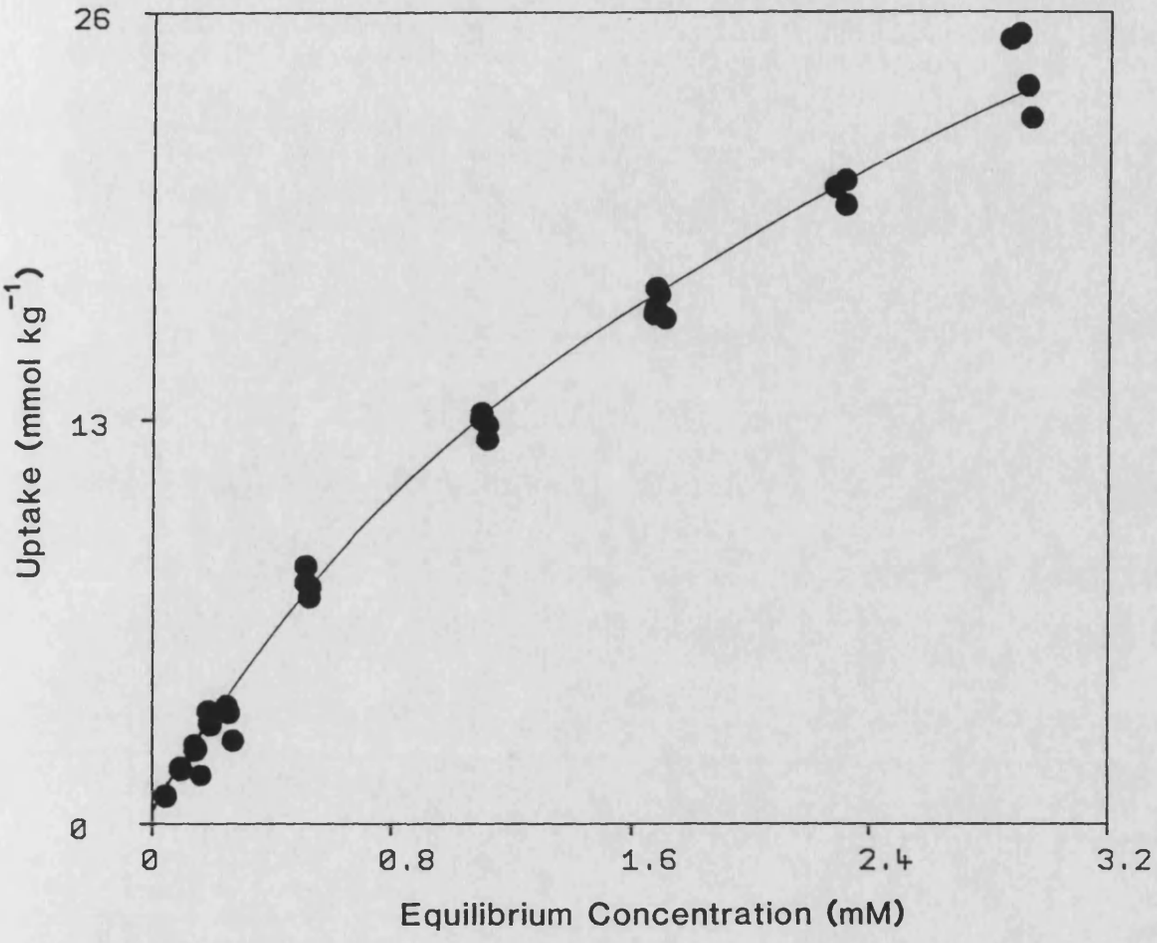


Figure 2.13. Sorption isotherm of propranolol HCl in buffer at 25°C with 1%C PHEMA.

due to such an interaction between the cationic propranolol ion and carboxyl groups in PHEMA, this is investigated in section 2.2.10.3. There are also the possibilities of hydrogen bonding or even hydrophobic bonding between the propranolol molecule and PHEMA which may act as stabilizing bonds, as seems to occur with chlorhexidine [250].

If such an interaction can be shown between propranolol HCl and PHEMA, it may explain the anomalous diffusion behaviour of propranolol HCl through PHEMA films found in section 2.2.8.

2.3.10 Effect of Methylation of the Free Carboxyl Groups in PHEMA

2.3.10.1 Methylation of PHEMA

The PHEMA discs initially became slightly opaque when the water was exchanged with ethanol prior to methylation, however the discs became transparent again after approximately 24 hours. The size of the discs changed after storage in ethanol, their diameters increasing together with an increase in their thickness. This process was reversed after methylation when the ethanol was exchanged with water, the polymers returning to their original size.

The production of diazomethane appeared successful, the activity of the pale yellow ethereal diazomethane was demonstrated by vigorous bubbling due to the evolution of nitrogen when a few drops of diazomethane were carefully transferred to a beaker of dilute acetic acid. After each methylation reaction the diazomethane solution was found to retain some activity as vigorous bubbling occurred when the residual solution was poured into dilute acetic acid, indicating the presence of excess diazomethane.

2.3.10.2 Assay of PHEMA for Free Carboxyl Groups

The carboxyl content of the samples analysed were as follows :

HEMA monomer : 110.2 $mmolkg^{-1}$ (S.D.=5.7)

Untreated PHEMA : 92.7 $mmolkg^{-1}$ (S.D.=9.3)

Methylated PHEMA : 3.6 $mmolkg^{-1}$ (S.D.=0.5)

The actual value for the HEMA monomer is probably less than that value produced in the assay as HEMA contains hydroquinone as a stabilizer. If both hydroxyl groups on the hydroquinone molecule react with potassium hydroxide in the titration, then the hydroquinone, at a concentration of 0.03%, will account for approximately 5 $mmolkg^{-1}$. There is probably a negligible amount of the stabilizer in the PHEMA as it would have been leached out in the preparation process (section 2.3.4.2).

The results appear to show the methylation of PHEMA to have been successful. The value for the carboxyl content of the methylated PHEMA are comparable to published values [251], however the values for both the untreated PHEMA and the HEMA monomer are greater than those found in previous studies. The values published in the literature for HEMA monomer is 2.2 $mmolkg^{-1}$, and for untreated PHEMA as 30 to 34 $mmolkg^{-1}$ depending on the batch of polymer. It was suggested that the difference between the monomer content and untreated PHEMA carboxyl content was due to acidic residues being generated in situ during polymerization [251]. In this present study however the values for monomer and untreated PHEMA are comparable, suggesting that PHEMA carboxyl content is related to monomer methacrylic acid content.

2.3.10.3 Uptake of Propranolol HCl by Methylated PHEMA

The sorption isotherms at 25°C for propranolol HCl in buffered solution with both methylated and untreated PHEMA are shown in figure 2.14. The isotherm for the methylated PHEMA shows that the propranolol HCl is taken up to a lesser extent than the untreated PHEMA, however the dramatic reduction in uptake that is seen with chlorhexidine digluconate and methylated PHEMA is not found in this study [251]. In the case of chlorhexidine uptake, a dramatic reduction is found, with only a small uptake compared to the untreated PHEMA, the residual interaction being related to the unreacted carboxyl residues.

The isotherm for propranolol HCl and methylated PHEMA (figure 2.14) shows that the carboxyl groups are only partly responsible for the interaction between propranolol HCl and PHEMA, one or more other interactions such as van der Waals forces, hydrogen bonding, or hydrophobic bonding may also be occurring, which in view of the functional groups present in propranolol HCl are all feasible. Support for this hypothesis comes from the values of partition coefficients calculated in section 2.3.8. The results indicate that the carboxyl content in PHEMA may be the predominant cause of interaction with some cationic drugs such as chlorhexidine, but as shown with propranolol HCl other interactions may be equally significant.

The pKa of the carboxyl groups in PHEMA should be carefully considered as it will determine the extent of ionisation of the groups at any given pH. The literature value for the pKa of methacrylic acid is 4.7 [265], however this may not be the value at the polymer surface since the incorporation into a polymer network of

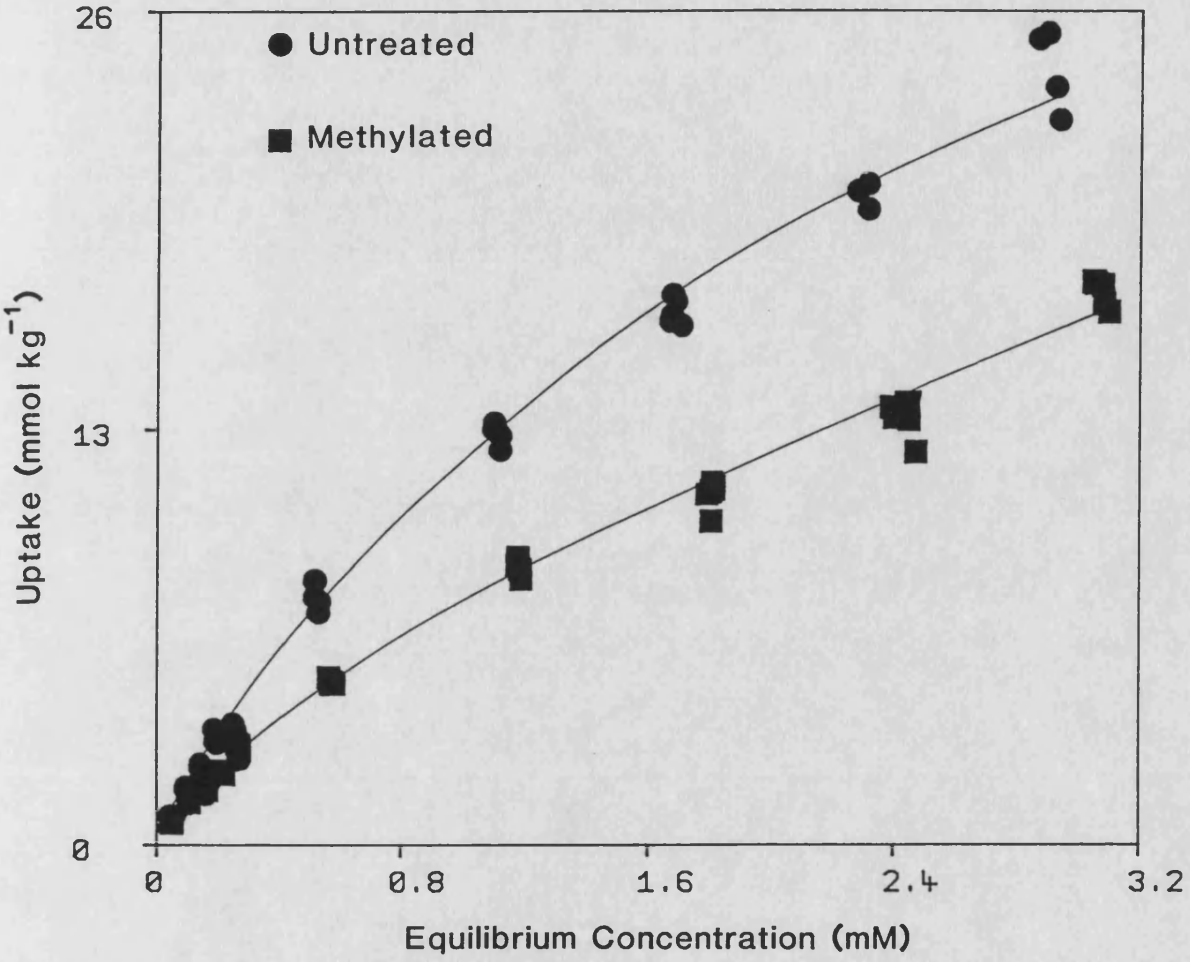


Figure 2.14. Sorption isotherms of propranolol HCl in buffer at 25°C with 1%C methylated and untreated PHEMA.

methacrylic acid may alter this pKa value. The pKa will also be affected by the nature of bound water, which would be expected to possess a lower dielectric constant than free aqueous water. Recent studies have indicated that the pKa of methacrylic acid incorporated into PHEMA may be as high as 6 to 7 [220], thus some caution is necessary in interpreting the results found in this study and previous studies with chlorhexidine digluconate as the pKa of the polymeric carboxyl groups was anticipated as being in the region of 3 to 4.

2.3.10.4 Diffusion of Propranolol HCL through Methylated PHEMA Films

The effect of methylating the carboxyl groups in PHEMA on the diffusion coefficient of propranolol HCl through crosslinked PHEMA films is shown in figure 2.15. Each pair of data points at any given crosslinker concentration were examined using a Students t-test to see if there was any statistically significant difference between the diffusion coefficient through methylated and untreated PHEMA, table 2.4 shows the significance levels calculated for each pair of points. These results suggest that the carboxyl groups in PHEMA appear to have an effect on the diffusion of propranolol HCl through PHEMA films when the predominant route of diffusion is via the pore mechanism (at low crosslinker content), however the carboxyl content does not seem to have any effect on the diffusion coefficient when a partition mechanism is in operation at higher crosslinker contents (section 2.3.8).

The results found are not what were initially anticipated, a possible cause of the anomalous diffusion behaviour of propranolol HCl through crosslinked PHEMA films was thought to be due to an

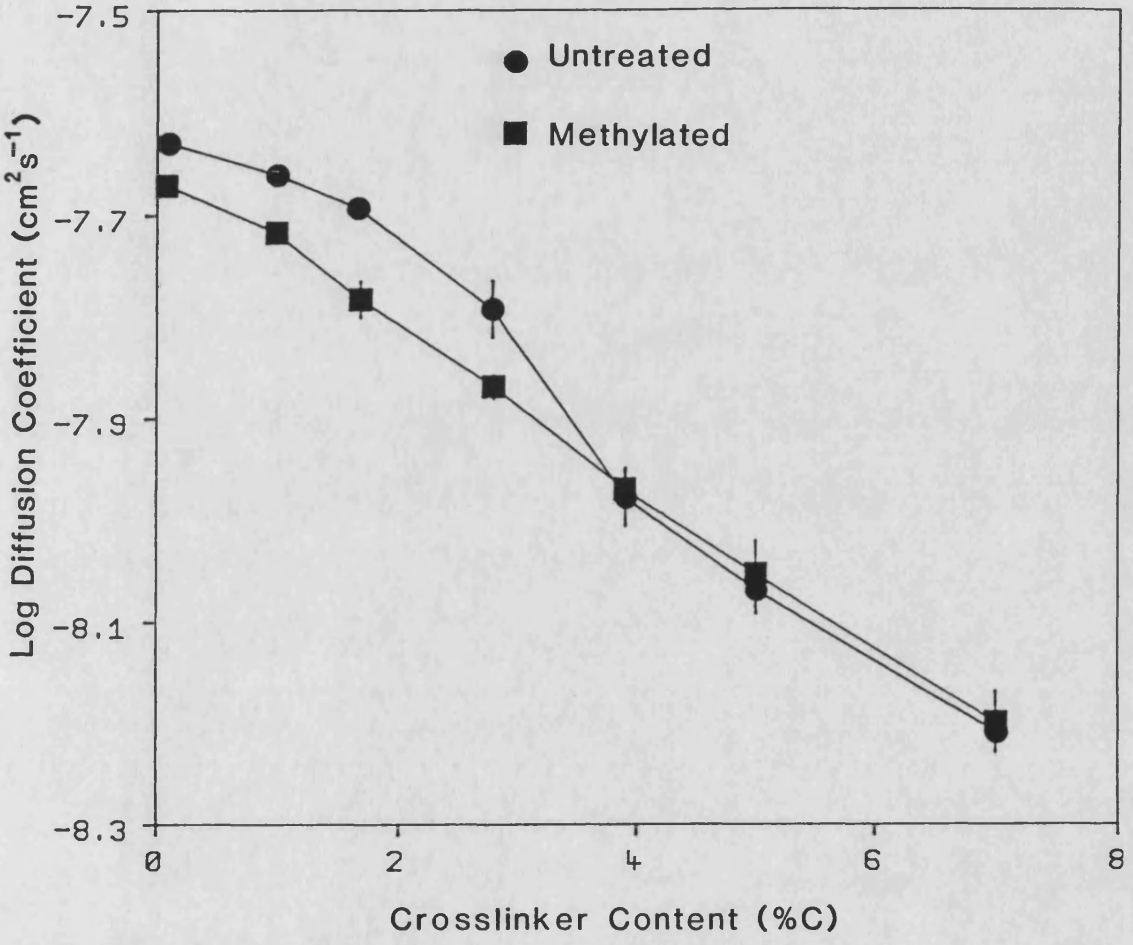


Figure 2.15. Effect of crosslinker content in PHEMA (methylated and untreated) on the diffusion coefficient of propranolol HCl through crosslinked PHEMA films (with standard error bars shown).

interaction between propranolol HCl and the carboxyl groups in PHEMA (section 2.3.8, section 2.3.9). If this was the cause then by methylating the carboxyl groups the diffusion coefficient of propranolol HCl through methylated PHEMA would be expected to increase at crosslinker contents less than about 3%C, rather than decrease as is found in this study. It was however found that the carboxyl groups in PHEMA are only partially responsible for the interaction between propranolol HCl and PHEMA (section 2.3.10.3), yet this still does not explain why methylation causes a decrease in diffusion coefficient. A small increase in the diffusion coefficient of some sort might still be expected rather than a decrease as was found, this would suggest that perhaps the decrease of diffusion coefficients caused by methylating the free carboxyl groups in PHEMA may be related to some other factor rather than just an effect on the interaction between propranolol HCl and PHEMA.

A possible explanation would be in terms of steric hindrance by the introduced methyl groups onto the polymer structure. At low crosslinker content the pore size is large enough to allow drug diffusion through the polymer by a pore mechanism, thus the introduction of methyl groups into the polymer structure might be expected to decrease the diffusion rate by steric hindrance. At high crosslinker contents where a partition mechanism is thought to occur, the introduction of methyl groups would not be expected to have such a drastic effect.

An alternative explanation for this effect would be if the presence of carboxyl groups caused PHEMA to swell, however it has been reported that significant swelling of PHEMA with small amounts of methacrylic acid does not occur unless the pH is greater than

about 6 [220]. Additionally the water content of PHEMA in pH 4.48 buffer determined in section 2.3.6 did not indicate any swelling greater than that which would be expected for crosslinked PHEMA.

If the carboxyl content in PHEMA is regarded as an impurity due to methacrylic acid, then the effect of this impurity on diffusion is to increase the diffusion coefficient of propranolol HCl through low crosslinked PHEMA films.

Table 2.4 Significance values calculated from a two-tailed Students t-test to examine whether there is any significant difference between the diffusion of propranolol HCl through methylated and untreated crosslinked PHEMA.

Crosslinker Content (%C)	Probability
0.1	.01
1.0	(>.01)
1.7	.01
2.8	.10
3.9	.84
5.0	.69
7.0	.70

Chapter 3

Controlled Transport of Propranolol HCl through Crosslinked PHEMA Using Constant Current Electrophoresis

3.1 Materials

Platinum foil, 10 mm x 10 mm x 0.025 mm, supplied by FSA Laboratories Supplies, Loughborough, U.K.

Platinum wire, 0.5 mm diameter, supplied by FSA Laboratories Supplies, Loughborough, U.K.

For all other materials used see section 2.1.

3.2 Methods

3.2.1 Apparatus and Techniques for Electrophoresis Studies

In this chapter the feasibility of an electrophoretically driven drug delivery device will be examined (section 1.3.9.2). The ability of this type of device to deliver a model ionised drug, propranolol HCl, to a delivery site in a fully controlled and predictable manner is investigated. The system examined in the present study is based on constant current electrophoresis, the polymer examined being crosslinked films of PHEMA. In designing the experiments, the main criterion was to demonstrate the full potential of this type of delivery device, in particular the possible application as a feedback controlled delivery system.

A schematic diagram of the electrophoretic model system used in this study is shown in figure 3.1, with a photograph of the actual apparatus in figure 3.2. The electrophoresis cell consisted of two equal compartments, and was based on the diffusion cell used in section 2.2.8 (figure 2.2). The same criteria used in the design of the diffusion cell were applied in the electrophoresis cell design. Each compartment of the cell has a capacity of approximately 220 ml, the dimensions of the cell being similar to that of the diffusion cell. The differences between the diffusion cell and electrophoresis

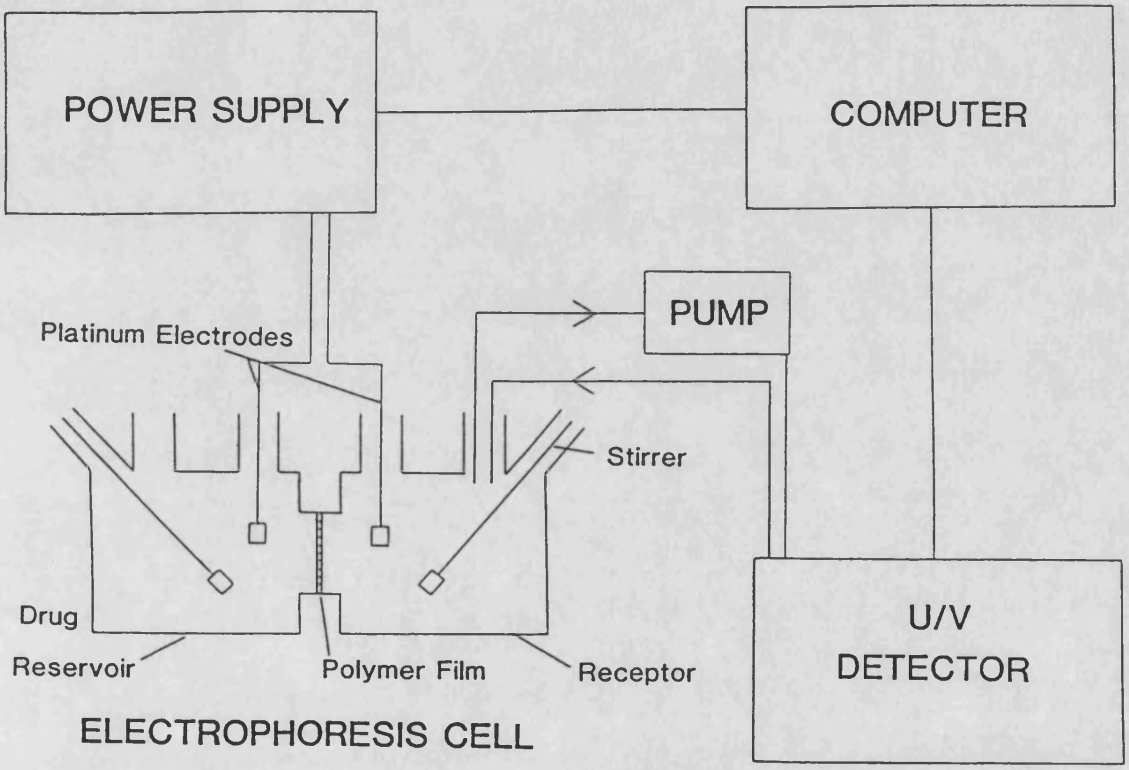
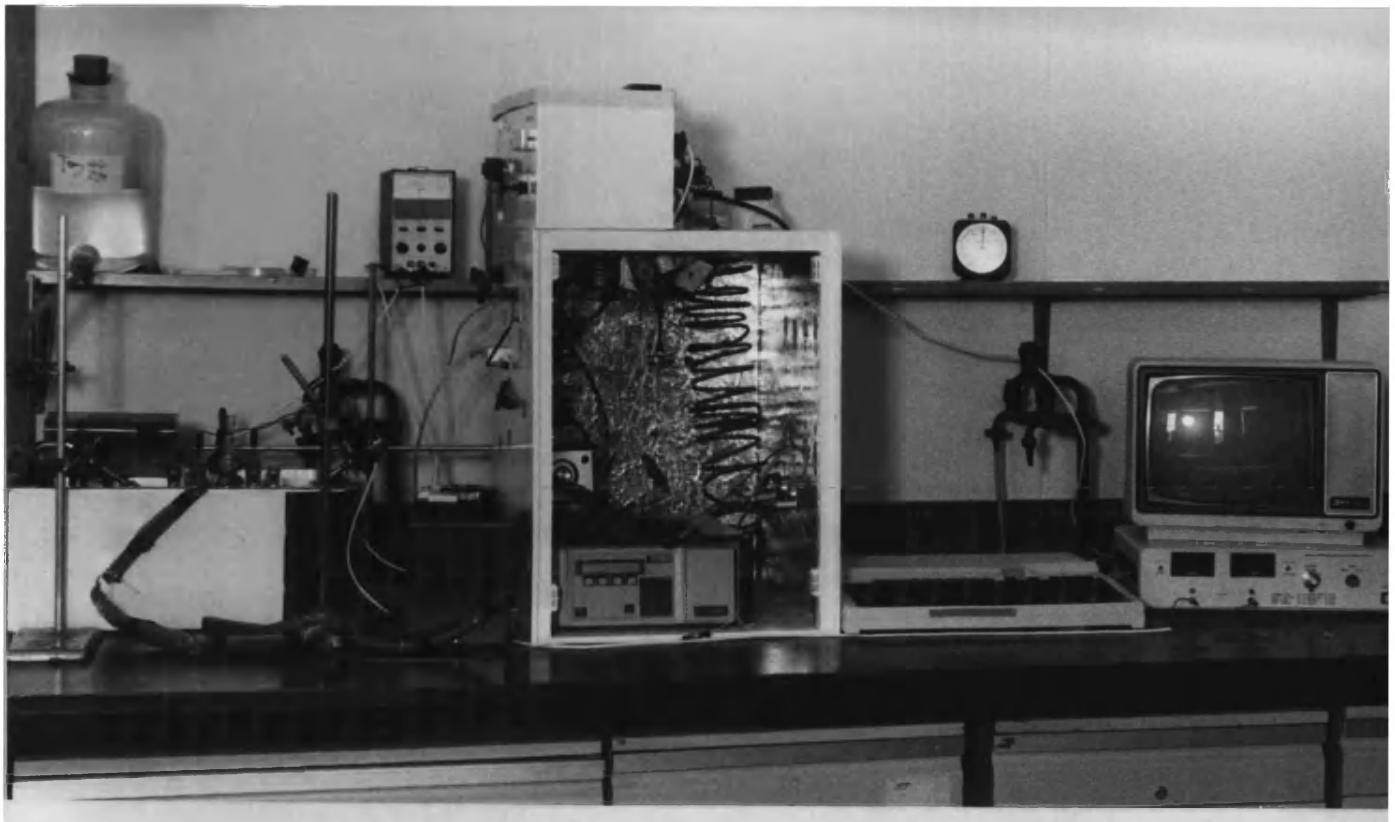


Figure 3.1. Schematic diagram of the apparatus used in electrophoresis studies.



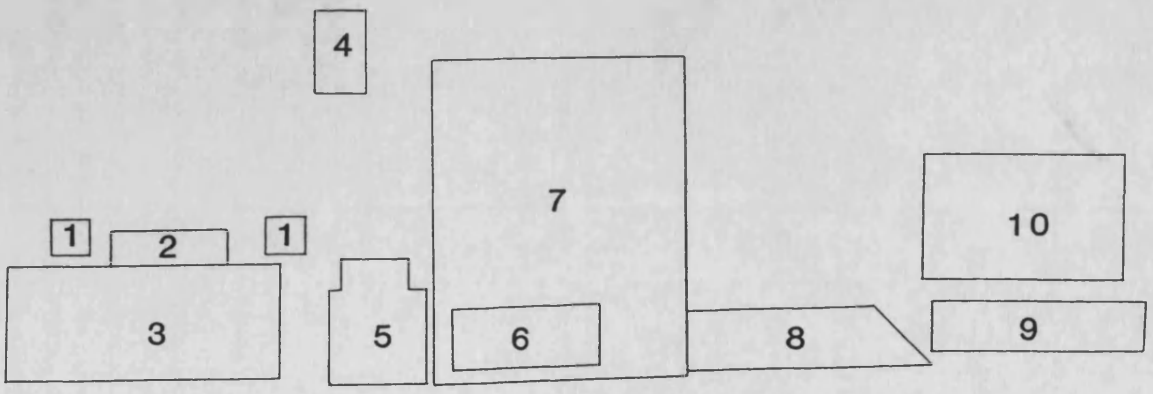


Figure 3.2. Photograph of the apparatus used in electrophoresis studies.

Key : 1 - stirrers, 2 - electrophoresis cell, 3 - thermostatted water bath, 4 - stirrer power supply, 5 - peristaltic pump, 6 - u.v. detector, 7 - temperature control cabinet, 8 - computer, 9 - constant current power supply, 10 - monitor.

cell being that the electrophoresis cell was designed with extra arms, one set to enable the insertion of electrodes, and the second set to allow stirring of the two compartments. The electrodes were fabricated from 1 cm^2 uncoated platinum foil, suspended on platinum wire (the use of uncoated platinum foil as opposed to platinized foil is discussed below in section 3.2.2). The separation of the two electrodes was maintained constant throughout all the experiments, at a distance of 8.5 cm. In all the experiments, unless otherwise stated, the receptor compartment electrode was the cathode.

The assembly of the electrophoresis cell and preparatory experimental details are very similar to those described for the diffusion cell in section 2.2.8. PHEMA films of the desired crosslinker content, having a thickness of approximately 0.1 cm were prepared as described in section 2.2.4.2, and discs of 4 cm diameter were cut. The discs were stored in pH 4.48 buffer at 4°C (appendix 2) prior to use. The interfacial ground glass joints of the electrophoresis cell were smeared with a thin layer of vacuum grease (Apiezon N). An annular sheet of polytetrafluoroethylene (PTFE), with a thickness of 0.08 cm and internal diameter of 4 cm was used as a liquid-tight gasket. The PTFE gasket was placed at the interface of the donor electrophoresis cell, and a disc of polymer film placed in its centre. The cell halves were then clamped tightly and provided an exposed area of polymer film of 6.16 cm^2. Into the receptor compartment was placed 210 ml of pH 4.48 buffer having an ionic strength of 0.039, prepared as described in appendix 2, and into the drug reservoir was placed 200 ml of pH 4.48 buffer. The two electrodes were then inserted into the appropriate arms of the electrophoresis cell (each electrode was always placed in the same

compartment for any given experiment to eliminate errors due to any differences in the electrode surface area). The whole cell was mounted in a specially constructed perspex stand and immersed in a water bath maintained at 25°C, unless otherwise stated. The compartments were stirred continuously using perspex stirrers driven by 12 volt motors at 330 rev min^{-1} (RS Components Ltd., Corby, U.K.), which were powered by a constant voltage power supply (E30/1 Farnell Instruments Ltd, Wetherby, U.K.).

The cell was allowed to equilibrate for 30 minutes, after which 20 ml of a stock propranolol HCl solution in acetate buffer was added to the reservoir compartment to give a final concentration of approximately 14 mM, unless otherwise stated. Timing was then started, and after 15 seconds a 10 ml sample was withdrawn from the reservoir compartment to give a measurement of the initial reservoir propranolol HCl concentration. This sample was analysed by u.v. spectrophotometry (section 2.2.1).

The receptor cell solution was pumped at a rate of 0.5 ml min^{-1} (Gilson Minipuls 2, Anachem, Luton, U.K.) into a flow-through u.v. detector (Gilson 116, Anachem, Luton, U.K.), and the outflow returned to the receptor cell. The u.v. detector, set to a wavelength of 288 nm, was connected to a microcomputer (BBC Master, Acorn Computers Ltd., Cambridge, U.K.), which collected the data at preprogrammed time intervals, usually of one hour. The computer was calibrated by passing a number of solutions of known concentration through the u.v. detector. The program used to calibrate the detector is shown in appendix 3. The computer, as well as recording the variation of receptor concentration with time was used to control the power supply. The constant current power supply, which was specially

designed and constructed in the School of Electrical Engineering at the University of Bath, was interfaced with the computer via the 1 MHz bus. The power supply could be controlled either manually or as in this study via the computer. Appendix 6 shows a block diagram of the power supply. The power supply could be set to two different current ranges, namely 0 to 25 mA and 0 to 2.5 mA, with the latter being selected for most experiments to ensure chemical stability as discussed in section 3.2.2 below. In addition to using software for controlling the magnitude of the current, the polarity of the electrodes could also be reversed, and the values of current (to two decimal places) and voltage (to one decimal place) read at any time by the computer. The computer could thus be programmed to control the power supply in a flexible manner. The effect of using electric currents on the transport of propranolol HCl was not examined until transport due to diffusion had become constant, which could be estimated from a plot of receptor propranolol HCl concentration against time. The computer was programmed to store values of absorbance, concentration, current, voltage and estimated drug delivery rate against time. The method used to calculate drug delivery rate is explained in section 3.2.11. The software written for this part of the study is shown in appendix 3, details of which will be further discussed below. At the end of any given experiment the data was stored onto floppy disc and analysed using a statistics package (INSTAT, University of Reading, U.K.), which enabled the effects of electrophoresis on the transport of propranolol HCl through crosslinked PHEMA films to be examined, as well as allowing the calculation of various parameters such as drug delivery rate.

At the end of each experiment the hydrogel thickness was measured (section 2.3.4.2). The electrodes were thoroughly rinsed in distilled water, followed by double distilled water. The electrodes were then stored in fresh double distilled water until required.

A control experiment was performed where no propranolol HCl was added to the drug reservoir, and the power supply turned on for several periods of four hours at a value of 15 mA. No significant baseline drift was found over a period of five days.

Control over the transport of propranolol HCl was achieved through PHEMA films using constant current electrophoresis in a model system, and this together with details of methodology used to study the effect of various parameters on the electrophoretically modulated release of propranolol HCl is described below. The experiments used were all based on the system described earlier in this section unless otherwise stated. In order to achieve the desired objectives, computer software was written to allow flexible control of both the power supply and data collection. The software written enabled a number of different experiments to be performed (appendix 3). Four types of experiment were possible with the software written, as shown by the first menu on lines 510-600. The first option, "keyboard control of power supply at any given time", was used mainly for diffusion studies in section 2.2.8, where the power supply was not required; it was also used in electrophoresis studies where it was required to turn the power supply power supply on or off at any given time. The second option, "power supply turned on and off at fixed times", allowed the power supply to be programmed to supply a given constant current between preprogrammed intervals. The third experimental option, "feedback control", was used to examine the

possible use of an electrophoresis device as part of a feedback controlled drug delivery device. This part of the program will be discussed in detail in section 3.2.11. The fourth option, "fixed release profile", enabled the power supply to be preprogrammed to turn on and off to any desired value or polarity several times in an experiment. A range of experiments are therefore possible with these options, however an experiment could be modified whilst in progress, and extra data collected if required. These available choices are shown in the second menu of the data collecting program (appendix 3) on lines 1090-1190. The options allow the power supply to be turned on or off to any desired value during an experiment, the polarity of the electrodes could also be reversed. The current, voltage, absorbance and associated concentration of propranolol HCl in the receptor could also be read at any time. The drug delivery rate into the receptor compartment at any given time could also be estimated (section 3.2.11). The program also allowed extra data to be collected at any time, therefore if any extra data points were required, or more frequent sampling then this option could be used.

3.2.2 Factors Affecting Degradation of Propranolol HCl During Electrophoresis

Despite the recent interest in the use of electrophoretically controlled drug delivery systems, no attention has been given to any potential degradation of active drug in the electrophoretic system [127,128,129], either due to electrode processes [266] or local heating effects in the polymer [118,267]. A previous study has commented on suspected drug degradation, but no detailed study was made [127]. This is a very important aspect of an electrophoretic

drug delivery device, as degradation of a drug could lead to inactivation of the drug or the production of irritant or toxic products. This would clearly be undesirable. In this present study the stability of propranolol HCl during electrophoresis will be investigated.

3.2.2.1 Use of Platinized Electrodes

In order to reduce polarization at platinum electrodes, a thin layer of platinum black is usually coated onto the electrode surfaces (section 3.3.8) [268]. The platinizing of electrodes was achieved by electro-deposition from a solution consisting of 2 g chloroplatinic acid and 0.02 g lead acetate in 100 ml of distilled water. The electrodes to be coated were placed in the solution and a 12 V power supply (E30/1 Farnell Instruments Ltd, Wetherby, U.K.) connected across the electrodes, the polarity of the electrodes being reversed every 30 seconds, this was repeated for 10 minutes. The electrodes were then placed in dilute sulphuric acid and the 12 V power supply was again connected for 10 minutes, the polarity being reversed every minute. The purpose of this was to remove traces of platinizing solution which are liable to adsorb onto the electrodes. The electrodes were then rinsed in warm distilled water several times, followed by double distilled water. The electrodes were subsequently stored in double distilled water prior to use.

3.2.2.1.1 Effect of High Electrophoretic Currents (0 - 25 mA)

Initial electrophoresis experiments were based on using currents in the range of 0 to 25 mA. The electrophoresis cell was assembled as detailed in section 3.2.1, with 1%C PHEMA and an initial drug

reservoir concentration of approximately 6 mM propranolol HCl was used. Once the delivery rate due to diffusion had become constant, the power supply was turned on to a current of 15 mA for a period of seven minutes. After approximately 17 hours the power supply was again turned on for a further seven minutes. The effect on the delivery rate of propranolol HCl into the receptor was examined.

3.2.2.1.2 Effect of Low Electrophoretic Currents (0 - 2.5 mA)

It was found that significant degradation of propranolol HCl occurred when a current of 15 mA was used in the model electrophoretic system (section 3.3.2.1.1). The power supply was modified as a result and the range scaled down by a factor of ten, to give a range of 0 to 2.5 mA. The electrophoresis cell was assembled as detailed in section 3.2.1, with 1%C PHEMA and an initial reservoir concentration of approximately 6 mM propranolol HCl. Once the delivery rate due to diffusion had become constant, the power supply was turned on to a current of 1 mA for one hour, then after approximately 19 hours the power supply was turned on to a current of 2 mA for a further hour, and the effect on propranolol HCl delivery rate into the receptor compartment examined.

3.2.2.1.3 Heating Effects in PHEMA

A significant but low level of degradation of propranolol HCl was still found in the current range of 0 to 2.5 mA (section 3.3.2.1.2). This degradation was probably due to one of two factors, either an electrode reaction was causing degradation, or alternatively a heating effect may be occurring in the PHEMA film during electrophoresis leading to thermal decomposition of

propranolol HCl. In the present study the temperature changes within PHEMA were examined during electrophoresis, to determine whether any significant heating was occurring, which might account for the small amount of degradation found during electrophoresis.

1%C PHEMA films were prepared as described in section 2.2.4.2, with one difference : a Type K nickel-chromium/nickel-aluminium thermocouple wire (RS Components Ltd., Corby, U.K.) was also placed into the polymerizing solution between the glass moulds. This required several experimental attempts to ensure that the thermocouple tip did not protrude from the hydrogel and was firmly embedded in the polymer film. A disc of 4 cm was then cut around the thermocouple tip using a scalpel. The electrophoresis cell was then assembled as in section 3.2.1, with the thermocouple wire being fed through the electrode arm in the reservoir compartment and connected to a digital temperature indicator (RS Components Ltd., Corby, U.K.). The initial reservoir concentration of propranolol HCl was 6 mM. Once the delivery rate due to diffusion had become constant, the power supply was turned on to a current of 15 mA for two hours, and any changes in temperature were noted.

3.2.2.2 Use of Uncoated Electrodes

Traditionally, electrophoresis studies using platinum electrodes have involved the use of platinized electrodes in order to reduce electrode polarization. The effect of platinizing the electrodes with a layer of platinum black is to increase the effective electrode surface area, and the platinum salt also acts as a catalyst for electrode processes [268,269]. In the present study uncoated platinum electrodes were used in the electrophoresis cell, in order to examine

whether the platinum black was responsible for, or catalysed the degradation of propranolol HCl found during electrophoresis using currents in the range of 0 to 2.5 mA (section 3.3.2.1.2). The electrophoresis cell was assembled as described in section 3.2.1, with 1%C PHEMA, and an initial reservoir concentration of approximately 6 mM propranolol HCl. Uncoated platinum electrodes were used in this experiment. Once the delivery rate due to diffusion had become constant, a current of 1 mA was passed for a period of 4 hours. The effect on propranolol HCl degradation was examined, together with the effect on the voltage required to maintain a constant current of 1 mA. The experiment was performed in duplicate.

3.2.3 Effect of Constant Current Electrophoresis on the Transport of Propranolol HCl through Crosslinked PHEMA Films

The effect of constant current electrophoresis on the transport of propranolol HCl through 1%C PHEMA films was examined. The electrophoresis cell was assembled as detailed in section 3.2.1, with an initial reservoir concentration of 14 mM, and once the delivery rate due to diffusion had become constant, a constant current was produced by the power supply for a period of 4 hours, then turned off again. The experiment was repeated for a range of currents from 0 to 2.5 mA, and changes in the delivery rate of propranolol HCl and in voltage were examined. Each experiment at a given current was performed in triplicate.

3.2.4 Effect of PHEMA Crosslinker Content on Electrophoresis

The majority of other experiments in this work were based on the use of 1%C PHEMA, where the transport of propranolol HCl through PHEMA is believed to be via a "pore mechanism" (section 2.3.8). In this part of the study the effect of using lower crosslinked PHEMA, 0.1%C on electrophoresis was examined, as was the effect of using a more highly crosslinked polymer, 7%C, where transport of propranolol HCl through the polymer is believed to be via a "partition mechanism" (section 2.3.8). The electrophoresis cell was assembled as described in section 3.2.1, with PHEMA of the appropriate crosslinker content. After the delivery rate due to diffusion had become constant, a constant current was produced by the power supply for a period of four hours, then turned off again. For any given crosslinker content the effect of different currents on electrophoresis was examined over the range 0 to 2.5 mA. Each experiment at a given current was performed in triplicate for 1%C and 7%C, and in duplicate for 0.1%C.

3.2.5 Effect of Buffer Ionic Strength on Electrophoresis and pH Changes During Electrophoresis

The effect of ionic strength on the electrophoretic delivery of propranolol HCl through crosslinked PHEMA film was examined in the present study. A range of ionic strengths was examined, from that of the acetate buffer (appendix 2), 0.039, to an ionic strength of 0.2. This range of ionic strengths was examined as it encompassed the ionic strength of plasma. The interest in this range stems from one of the envisaged applications of the electrophoretic delivery device as an implant system (section 1.3.9.2), where the ionic strength of the environment may have an effect on the device. The ionic strength

of plasma was approximated from the concentration of the principal ions in plasma [270], the value estimated as 0.149.

The electrophoretic cell was assembled as described in section 3.2.1, using 1%C PHEMA. Once the delivery rate due to diffusion had become constant, a constant current of 2.4 mA was produced for a period of 4 hours. The experiment was repeated for a range of ionic strengths, the reservoir concentration of propranolol HCl being kept constant at 14 mM for all the experiments.

A range of ionic strengths of the acetate buffer were prepared by adding a known quantity of sodium chloride to the acetate buffer. Prior to use, the sodium chloride was dried over phosphorous pentoxide in a vacuum oven at 80°C for 48 hours to remove traces of water. The effect of ionic strength on the pH of the buffer was estimated by taking account of activity effects (using the Debye-Huckel equation) on the pH of the buffer, calculated using the Henderson-Hasselbalch equation [271]. The calculated change in pH caused by increasing the ionic strength of the buffer to 0.2 is approximately 0.06 pH units. This change in pH was thought to be insignificant as far as electrophoresis is concerned since the pKa of propranolol HCl is 9.5 [253]. The actual pH of the various ionic strength buffers was measured and compared to the theoretically calculated values. A u.v. scan of the 0.039 and 0.20 ionic strength buffer was performed between the wavelengths of 200 and 400 nm (section 2.2.1) in order to examine whether the addition of sodium chloride affected the u.v. absorbance of the acetate buffer. A series of stock solutions were prepared, containing propranolol HCl in acetate buffer of the same concentration as those being investigated in this study but having a range of ionic strengths. This was done so

that the effect on ionic strength of adding stock propranolol HCl at the start of an experiment was constant.

As well as examining the effects of ionic strength on electrophoresis, the changes in pH that occurred during electrophoresis were also examined for each experiment. A combination electrode, 0.7 cm in diameter and 10 cm long (Russell CTWL/B14, Russell pH Ltd., Fife, U.K.) was used in this study. The electrode could be inserted into either compartment of the electrophoresis cell periodically during an experiment to measure the pH (appendix 2).

3.2.6 Effect of Temperature on Electrophoresis

The effect of temperature on the electrophoretic transport of propranolol HCl through 1%C PHEMA was examined. The electrophoretic cell was assembled as described in section 3.2.1, using a film consisting of 1%C PHEMA and an initial reservoir concentration of 14 mM Propranolol HCl. Once the delivery rate due to diffusion had become constant, a constant current was produced by the power supply for a period of 4 hours. The experiment was repeated in triplicate for a range of currents from 0 to 2.5 mA, and changes in the delivery rate of propranolol HCl determined. This series of experiments was repeated at both 31°C and 37°C, data for 25°C conditions having already been obtained in section 3.2.3.

3.2.7 Effect of Reservoir Propranolol HCl Concentration on Electrophoresis

The effect of varying the concentration of propranolol HCl in the reservoir compartment on the drug delivery rate produced by an electrophoretic current of 1 mA was examined in the present study.

Varying the reservoir concentration would be expected to alter the delivery rate across the PHEMA film as a result of diffusion effects as well as effects on electrophoretic control. The electrophoretic cell was assembled as described in section 3.2.1 using a film of 1%C PHEMA. Usually the volume of buffer placed in the reservoir compartment was 200 ml, with 20 ml of stock propranolol HCl added at the start of an experiment. However, in the present study, the volume of buffer placed in the reservoir was varied so that on addition of an appropriate volume of stock propranolol HCl solution necessary to make the volume up to 220 ml, a range of reservoir propranolol HCl concentrations could be obtained from 1 to 55 mM. The final concentration of the reservoir was determined as described in section 3.2.1 by removing a 10 ml sample from the reservoir for assay. Once the delivery rate due to diffusion had become constant, a constant current of 1 mA was produced by the power supply for a period of 4 hours. The experiment was repeated for a range of reservoir concentrations.

3.2.8 Factors Affecting the Resistance Across PHEMA Films During Electrophoresis

The effect of a number of factors on the resistance of the model electrophoretic system were examined. The voltage across the platinum electrodes was measured using a voltmeter (Weston 6100, supplied by RS Components Ltd., Corby, U.K.). The computer supply only measured voltages in the range 0 to 25 V to one decimal place which was not considered of a high enough precision for the present study. The resistance across the electrodes was measured at the end of each experiment. A range of currents from 0 to 2.5 mA was applied to the

system, and for any given current, the voltage measured after 10 minutes. From these results the resistance could be calculated by determining the gradient of a plot of voltage against current, using Ohm's law :

$$V = I.R$$

V = Voltage (V)

I = Current (A)

R = Resistance (Ω)

The results were used to give an indication of the influence of various factors on the electrophoretic system, such as temperature, ionic strength and crosslinker content, as well as to provide an indication of the power requirements of an electrophoretic device.

3.2.9 Effect of Reversing Electrode Polarity

The effect of electrophoresis on the transport of propranolol HCl through 1%C PHEMA films caused by reversing the polarity of the platinum electrodes was examined. Earlier experiments showed that constant current electrophoresis could be used to modulate the delivery of propranolol HCl across films of crosslinked PHEMA (section 3.3.3), however a basal delivery rate due to diffusion was always apparent. The aim of this part of the study was to investigate whether such basal drug delivery due to diffusion could be reduced or even halted by electrical intervention. A previous study has examined the modification of polymer formulation to reduce basal drug delivery due to diffusion (see section 3.3.3) [127]. The electrophoretic cell was assembled as described in section 3.2.1, with 1%C PHEMA and an initial reservoir concentration of 14 mM propranolol HCl. Once the delivery rate due to diffusion had become constant the computer was

programmed to turn the power supply alternately on and off continually at fixed intervals. The power supply was turned on for six hour periods, and off for seven hour periods. The electrode polarity was reversed from that which was used in the rest of this work, so that the receptor electrode was now the anode. On each subsequent period of electrophoresis, the computer was programmed to increase the current in small steps, and the effect on delivery rate examined.

3.2.10 Effect of Electrophoresis on Diffusion Lag Time

In all experiments designed to investigate controlled drug transport through crosslinked PHEMA films using constant current electrophoresis, electrophoresis studies were only commenced after the delivery rate due to diffusion had become constant (section 3.2.1), for reasons discussed in section 3.3.3. One disadvantage of using such an approach was that a lag time greater than 24 hours was necessary before any studies could be performed. This long lag time could in many cases be unacceptable from a therapeutic point of view. However other types of drug reservoir devices such as the transdermal patch, TTS-scopolamine, contain a priming dose in the contact adhesive which partly compensates for the time required for steady state conditions to be established [26]. Previous studies on the use of electrophoretic drug delivery devices [127,128] have not considered whether any significant differences exist between the effects of electrophoresis during the time period before steady state diffusion has been attained and the effects of electrophoresis once steady state diffusion has been attained. In these previous studies the effects of electrophoresis appear to have been examined prior to

steady state diffusion being attained (section 3.3.3). The aim of this part of the study was to examine the significance of attainment of steady state diffusion on electrophoretically controlled drug delivery. The electrophoresis cell was assembled as described in section 3.2.1, with 1% PHEMA and an initial reservoir concentration of 14 mM propranolol HCl. An electrophoretic current of 2 mA was applied for a period of 8 hours starting approximately 20 seconds after initiation of the experiment. The effect of the electrophoretic current on drug delivery was examined.

3.2.11 Delivery of Propranolol HCl through Crosslinked PHEMA by a Programmable Feedback Electrophoresis System

The most innovative and therapeutically significant aspect of an electrophoretic drug delivery device is its potential for use as a feedback driven device (section 1.3.9.2). In this part of the study, the feasibility of delivering propranolol HCl through crosslinked PHEMA by a programmable feedback electrophoresis system will be examined. A feedback system is envisaged as comprising of the electrophoretic device, and a transducer capable of detecting therapeutically significant changes effected by the drug. Depending on the relationship between the transducer output and a pre-set level, the output of the electrophoretic device should be capable of being altered appropriately until the transducer detects the desired effect and outputs a signal corresponding to the pre-set level. In the present study the u.v. detector was used as a model transducer, and the computer was used to estimate the drug delivery rate at any given time. The computer was programmed to adjust the output of the constant current power supply in response to the output level from

the u.v. detector so that a desired preprogrammed drug delivery rate could be achieved for a fixed period of time.

The electrophoretic cell was assembled as described in section 3.2.1 using a film of 1%C PHEMA and an initial reservoir concentration of 14 mM propranolol HCl. The computer was programmed to allow for feedback control of propranolol HCl over a period of 8 hours commencing at 35 hours and the effects on drug delivery rate were examined. There were two major problems in designing the feedback experiments: firstly, obtaining an appropriate assessment of the delivery rate at any given time; secondly, having determined the delivery rate, selecting an appropriate method of adjusting the power supply output in order to obtain the desired delivery rate.

The method used initially to estimate the delivery rate of propranolol HCl into the receptor was based on setting the computer to collect data from the detector at intervals of three minutes. The program then selected the ten most recent data points covering the previous 30 minutes, from which the computer estimated the delivery rate using linear regression analysis. The procedure used to perform this calculation, PROCz, can be found at line 2410 in the data collecting program shown in appendix 3. The initial feedback experiments were based simply on programming the computer with a desired delivery rate, then during the period of feedback control the computer compared the pre-set value corresponding to the desired delivery rate to the value obtained from linear regression analysis. If the estimated delivery rate was less than the pre-set value, the power supply was turned on to provide a current of 2.4 mA. If the estimated value exceeded the desired value the power supply was switched off. A series of experiments were performed using this

method of feedback, with the computer programmed to deliver propranolol HCl at one of three delivery rates ; 0.6 mg/h, 0.8 mg/h, 1.0 mg/h.

The method by which the delivery rate was estimated using data from the detector was also examined. The effect of using different sample sizes and frequencies on the feedback control was examined when a desired delivery rate of 0.8 mg/h was programmed into the computer. The sampling routine used initially was based on 10 samples at three minute intervals, other routines examined were 10 samples at 5 minute intervals, 5 samples at 10 minute intervals and 5 samples at 6 minute intervals.

A second method of adjusting the power supply during feedback control was also examined. In section 3.3.3 a linear relationship was determined between current and drug delivery rate for 1%C PHEMA, thus an equation relating current and delivery rate could be obtained. For example, if a particular delivery rate was measured and a higher or lower delivery rate was required, then the change in current needed to obtain the new delivery rate could be calculated using the known relationship between current and delivery rate. The procedure PROCbi, found at line 2660 of the data collecting program (appendix 3), is the routine used to adjust the power supply according to this method. The computer was programmed to compare the estimated delivery rate (based on 10 samples at 5 minute intervals) to the required delivery rate, then to change the current appropriately to that which would be expected to give the desired delivery rate. The procedure was written so that the highest current output was 2.4 mA and the lowest 0 mA. The experiment was performed using a single programmed rate of 0.8 mg/h.

3.3 Results and Discussion

3.3.1 Apparatus and techniques for electrophoresis studies

A typical plot obtained during an electrophoresis experiment showing the effect of an applied current on the transport of propranolol HCl through crosslinked PHEMA is shown in figure 3.3. The example shown is for 1%C PHEMA with a propranolol HCl reservoir concentration of 14 mM, obtained using a current of 2.3 mA applied for a period of four hours. Samples were taken at hourly intervals, except during electrophoresis when samples were taken every twenty minutes. Application of a current had an almost immediate effect, increasing the drug delivery rate into the receptor significantly. On removal of the current, the drug delivery rate dropped significantly. The data for each experiment was analysed to check for linearity of the drug delivery rate during electrophoresis. For each experiment the diffusion coefficient of propranolol HCl through PHEMA (without electrophoresis) was calculated from the lag time and film thickness (appendix 1), in order to check the integrity of the system. The initial reservoir concentration was also calculated.

3.3.2 Factors Affecting Degradation of Propranolol HCl During Electrophoresis

3.3.2.1 Use of Platinized Electrodes

3.3.2.1.1 Effect of High Electrophoretic Currents (0 - 25 mA)

The effect of application of a constant current of 15 mA on the delivery rate of propranolol HCl into the receptor compartment was dramatic, each time a current was supplied a rapid and large rise in the delivery rate occurred compared with that of due to diffusion prior to electrophoresis (figure 3.4). However, during

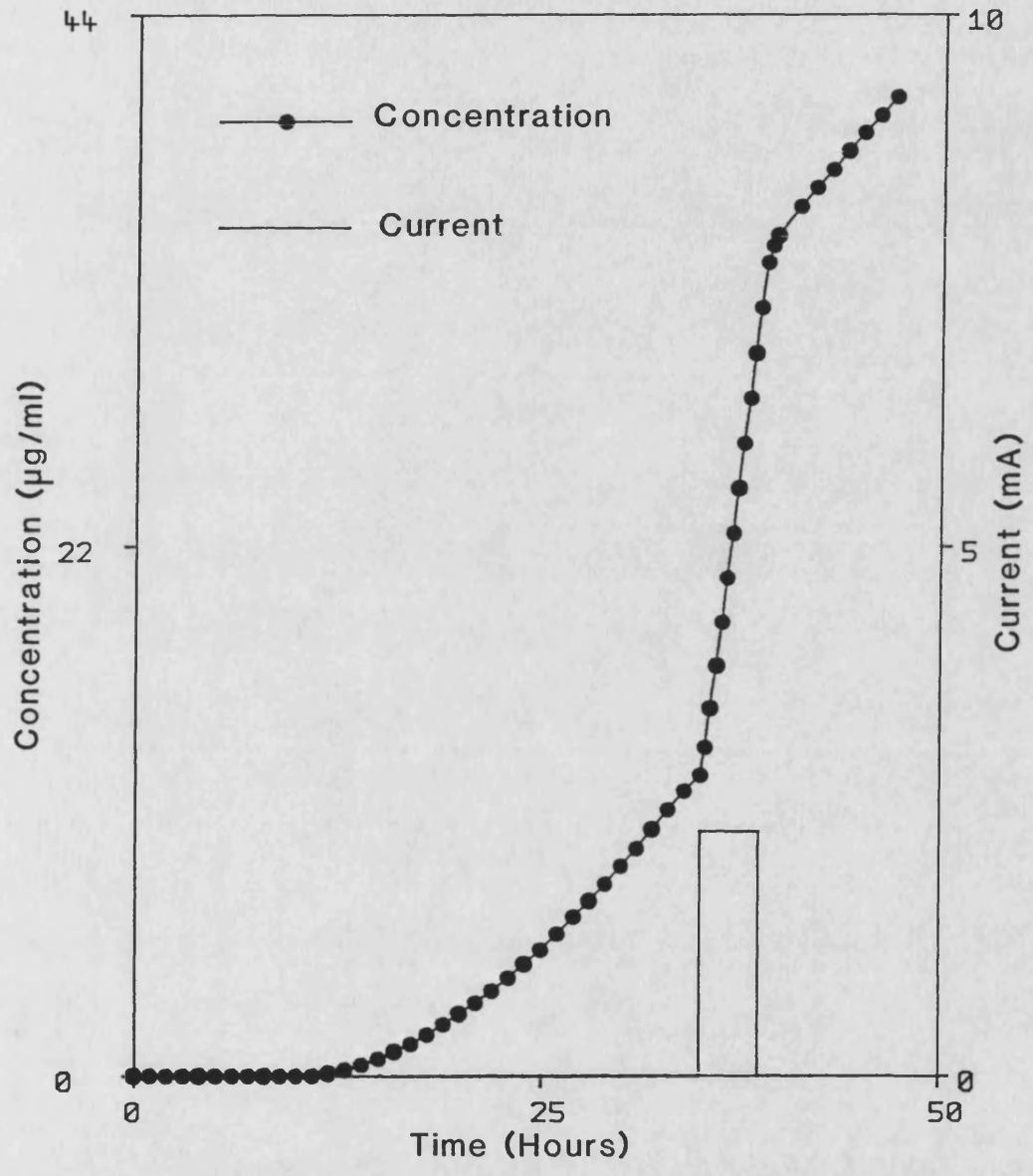


Figure 3.3. Effect of applied current on the transport of propranolol HCl through 1%C crosslinked PHEMA film.

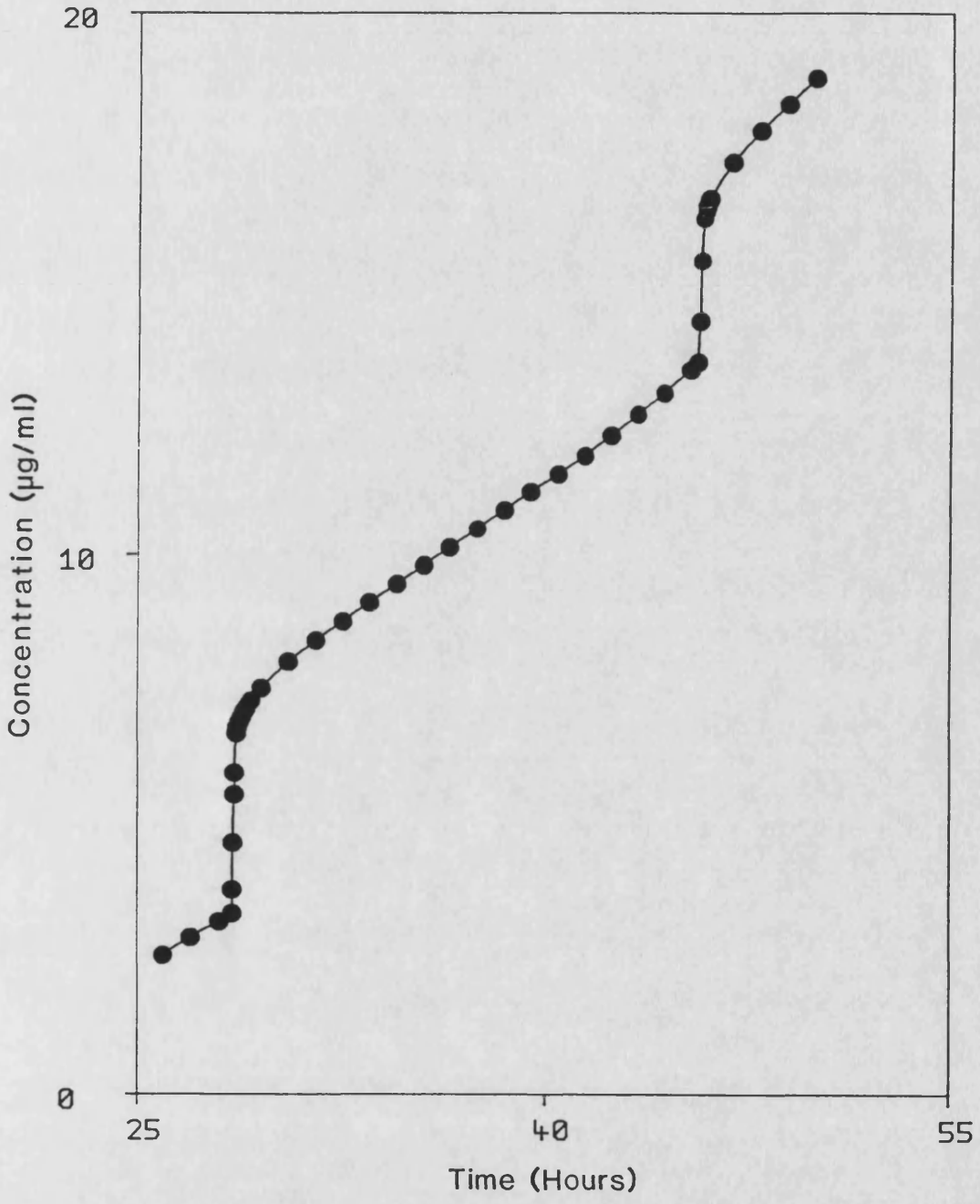


Figure 3.4. Effect of applying an electrophoretic current of 15 mA on two separate occasions (each time for a period of seven minutes) on the transport of propranolol HCl through 1%C PHEMA film.

electrophoresis there appeared to be visible electrolysis of water, with bubbles being evolved at the electrodes: assumed to be hydrogen at the cathode and oxygen at the anode. At the end of the experiment, the solutions in both reservoir and donor compartments were coloured brown. A control experiment (section 3.2.1) showed no discolouration in the absence of propranolol HCl from the reservoir compartment. This would suggest that the discolouration was not caused by material coming off the electrodes or being leached from the polymer, but more likely to be due to degradation of propranolol HCl as a result of application of a current. Samples of the reservoir and receptor compartments were taken at the end of the experiment and analysed for degradation of propranolol HCl using HPLC assay (section 2.2.2). The assay showed that although only 5.0% degradation of propranolol HCl had occurred in the reservoir, 45.6% of the delivered propranolol HCl had degraded in the receptor. Thus even though a constant current of 15 mA had a significant effect on the drug delivery rate, a significant level of drug degradation had also occurred, thus making the use of such a high current unsuitable for modulating the delivery of propranolol HCl.

3.3.2.1.2 **Effect of Low Electrophoretic Currents (0 - 2.5 mA)**

The effect on the delivery rate of propranolol HCl into the receptor compartment using lower currents was significant, but not as great as that produced by a current of 15 mA (section 3.3.2.1.1). The delivery rate produced by the 2 mA current was greater than that produced by the 1 mA current, which was in turn greater than the delivery rate produced by diffusion alone (figure 3.5). A quantitative study of the effect of various constant currents on drug

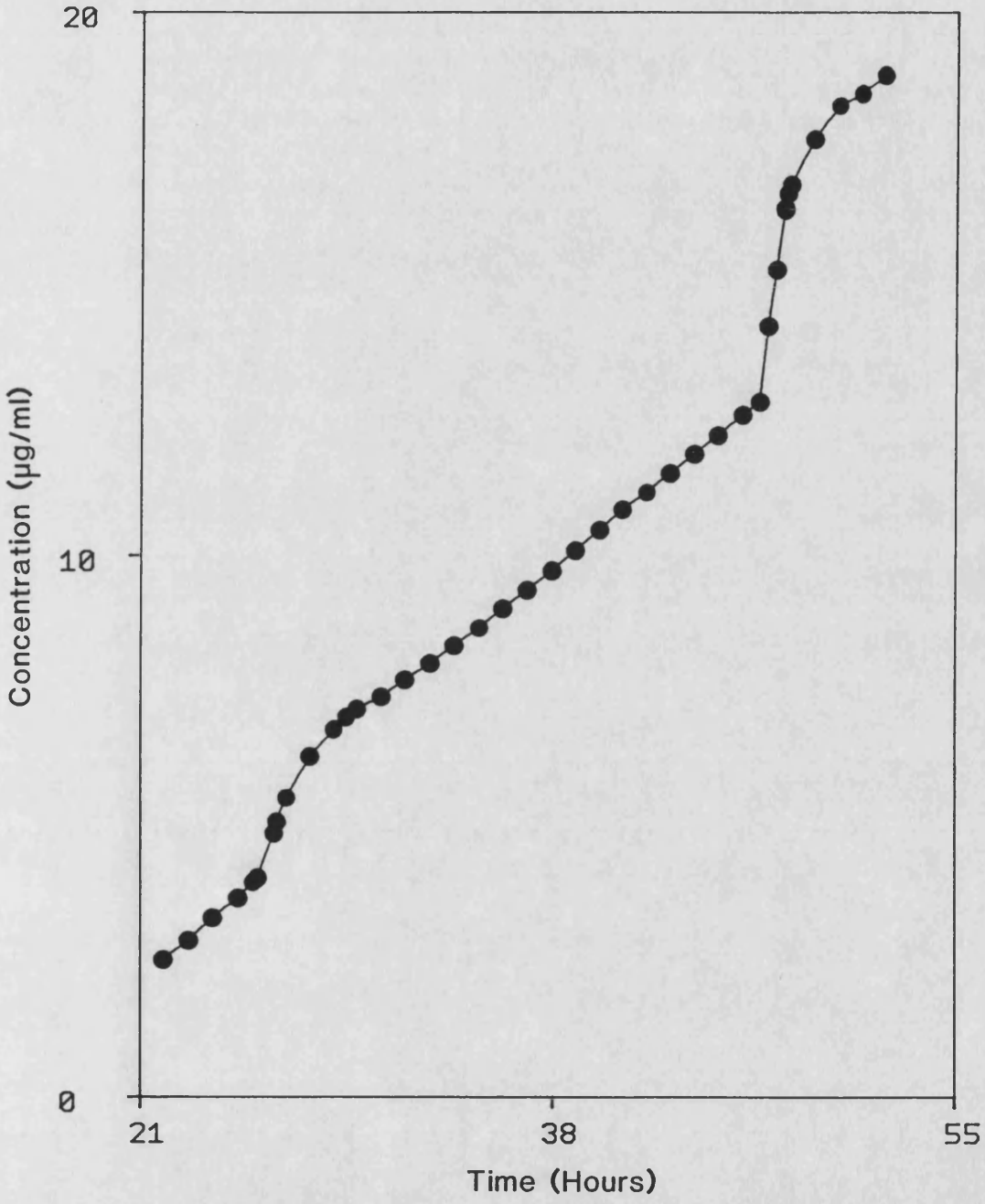


Figure 3.5. Effect of applying a current of 1 mA for a period of one hour, followed 19 hours later by the application of a current of 2 mA for one hour, on the transport of propranolol HCl through 1%C PHEMA film.

delivery rate is found in section 3.2.3. No visible electrolysis of water was found with the lower currents, however there was still the presence of a slight brown discolouration after the electrophoresis. Samples of the reservoir and receptor compartments were removed at the end of the experiment and analysed for degradation of propranolol HCl using HPLC assay (section 2.2.2). The assay showed a 1.2% degradation of propranolol HCl in the reservoir, and that 7.8% of the delivered propranolol HCl had degraded in the receptor. Thus using the lower currents a significant effect on the drug delivery rate can still be produced, with drug degradation much less of a problem, though still present.

3.3.2.1.3 Heating Effects in PHEMA

Use of a thermocouple embedded in PHEMA films did not reveal any significant heating effects during electrophoresis, the digital temperature indicator was found to remain constant at 25°C throughout electrophoresis. Visible gas evolution occurred from the electrodes and visible darkening of the solutions occurred in this period. Heating effects during polyacrylamide electrophoresis, when used as an analytical technique, are well known [118,267], however, in this use of electrophoresis very high voltages and currents are applied across polyacrylamide discs. It was therefore considered that the reason that no localized heating effects were found in the present study was due to the very low currents used, perhaps combined with efficient heat transfer. It was therefore concluded that heating effects were probably not involved in producing the degradation of propranolol HCl found during electrophoresis in the system under investigation.

3.3.2.2 Use of Uncoated Electrodes

A significant effect on the delivery rate of propranolol HCl into the receptor compartment was still found when using uncoated platinum electrodes (figure 3.6). However, the voltage required to provide the current increased by approximately 1 volt in comparison with that required in the system using platinized electrodes (details on the magnitude of current and voltages are found in section 3.3.3). No brown discolouration was found after electrophoresis. Samples of the reservoir and receptor compartments were taken at the end of both experiments and analysed for degradation of propranolol HCl using HPLC assay (section 2.2.2). The assay showed that no detectable degradation had occurred after a constant current of 1 mA had been flowing for 4 hours. Thus it would appear that the layer of platinum black on the electrodes may be responsible for the previously detected degradation of propranolol HCl (section 3.3.2.1.2). Degradation may still be occurring, but at a much less significant level. Thus significant degradation of propranolol HCl can be avoided during constant current electrophoresis provided that uncoated platinum electrode are used. In the rest of this work uncoated platinum electrodes were used. Several checks on degradation were performed on later experiments using a range of currents where the initial propranolol HCl reservoir concentration was 14 mM as opposed to 6 mM in this degradation study. No detectable degradation was found in any of these experiments.

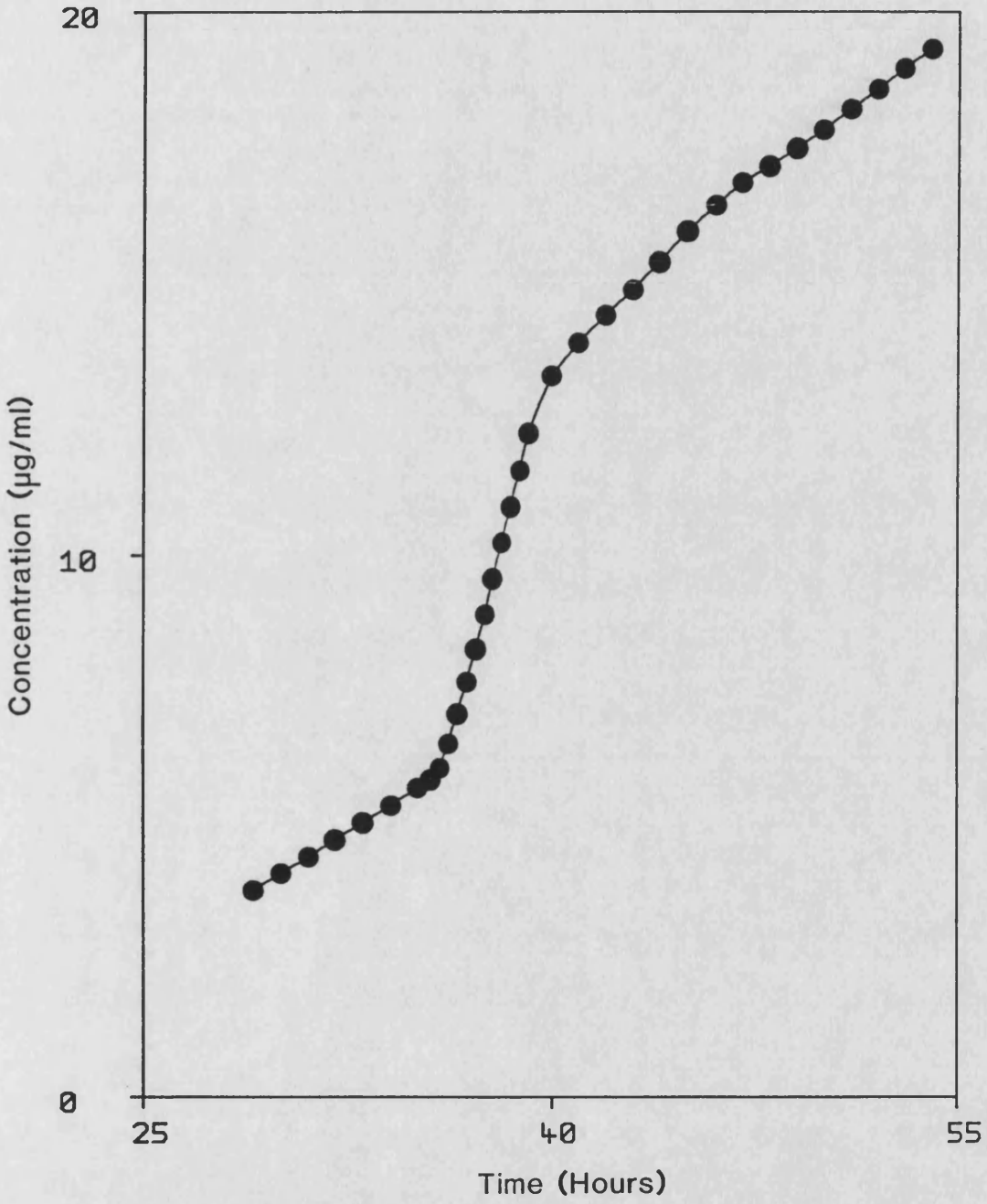


Figure 3.6. Effect of an applied current of 1 mA for four hours on the transport of propranolol HCl through 1%C PHEMA film.

3.3.3 Effect of Constant Current Electrophoresis on the Transport of Propranolol HCl through Crosslinked PHEMA Films

Figure 3.3 shows the relationship between propranolol HCl receptor compartment concentration and time using a 1%C PHEMA film, showing the effect that an electric current of 2.3 mA has on the delivery rate of propranolol HCl into the receptor when applied for a period of four hours. This type of profile is representative of those obtained with all the currents examined in the range 0 to 2.5 mA, the magnitude of the change in drug delivery rate was found to vary with current. Examples of the types of profiles obtained in electrophoresis experiments may be found in appendix 4. A significant effect on the delivery rate of propranolol HCl was produced by the application of a current, the effect of a current being to increase the delivery rate over that produced by diffusion alone.

Previous studies on electrophoretic devices using polyacrylamide [127,128] have indicated that when no electrophoretic current is applied, no measurable drug delivery occurs, thus drug delivery will only occur during electrophoresis. This type of control over drug delivery rate would be highly desirable, however these results should be viewed with caution as these models may not be realistic. In the case of Lescure et al. [128], no details of the concentrations of drugs used or polymer thickness are given, so no comments can be passed. However, in work reported by Kumar [127], the absence of drug delivery due to diffusion may be explained. There are two factors to be considered. The first and most important concerns the lag period of diffusion. In permeability studies it was found that with a polyacrylamide disc of 1.5 mm thickness, taking hyoscine methyl chloride as an example, a lag time of approximately two hours was

found [127]. In the electrophoresis studies which were over a period of approximately 90 hours, the polyacrylamide discs used were of a thickness of 10 mm. On the basis of these figures, with a 10 mm disc, if diffusion was the only process occurring then it could be expected that a lag period of approximately 88 hours would exist, since lag time is proportional to the square of polymer thickness (appendix 1). Drug delivery produced by electrophoresis before the calculated diffusion lag time is not surprising, since as was shown in this work lag times can be interrupted using electrophoresis, the lag time apparently resuming after cessation of electrophoresis (section 3.3.10). Examining electrophoretic effects during this lag time may lead to misleading interpretation of results, since as well as examining the effect of electrophoresis, there will be the concomitant changes in concentration that occur during the diffusion lag time. The second and probably less important factor is that significant depletion of the reservoir compartment occurred in the reported study during electrophoresis (leading to a significant build-up in the receptor). This factor may also contribute to the fact that no drug delivery due to diffusion was detected in that study. Diffusion controlled drug delivery is dependent on the maintenance of a concentration gradient (appendix 1), however if this concentration gradient decreases, as would appear to be happening in the previous study, then there will be a gradual decrease in the driving force of diffusion, resulting in a continual decrease in the amount of drug being delivered by diffusion, and possibly resulting in a diffusion delivery rate too small to be detected. In the present study, high concentrations of propranolol HCl were used in the reservoir and sink conditions prevailed throughout the

experimental time (the receptor concentration never exceeded 2% of the reservoir concentration during experiments), also electrophoresis studies were commenced only after the delivery rate due to diffusion had become constant. This model was thought to be more realistic of an electrophoretically controlled drug delivery device where a drug loaded reservoir would probably be used. The maintenance of sink conditions explains why diffusion controlled drug delivery was found in this study.

The lag time before which an applied current had a significant effect on the drug delivery rate was found to be less than five minutes (see figure 3.3 and appendix 4). Only one previous study was found on the use of electrophoretic drug delivery devices that commented on the magnitude of this lag time [128], where it was reported that using crosslinked polyacrylamide discs a lag time of about one hour occurred before any noticeable effect on drug delivery rate was produced. The work was carried out using a constant current power supply to control the delivery of bovine serum albumin. No details of the polyacrylamide gel thickness were given, however this long lag time would suggest that the polymer thickness was considerably greater than that examined in the present study.

The drug delivery rate during electrophoresis was found to be linear (zero-order) for all values of current examined; the value of the delivery rate of propranolol HCl into the receptor compartment was determined from the change in concentration that occurred with time during electrophoresis. The relationship between propranolol HCl delivery rate and current was found to be linear, figure 3.7 shows this relationship (standard error bars were too small to be shown). Linear regression analysis of the line yielded the following

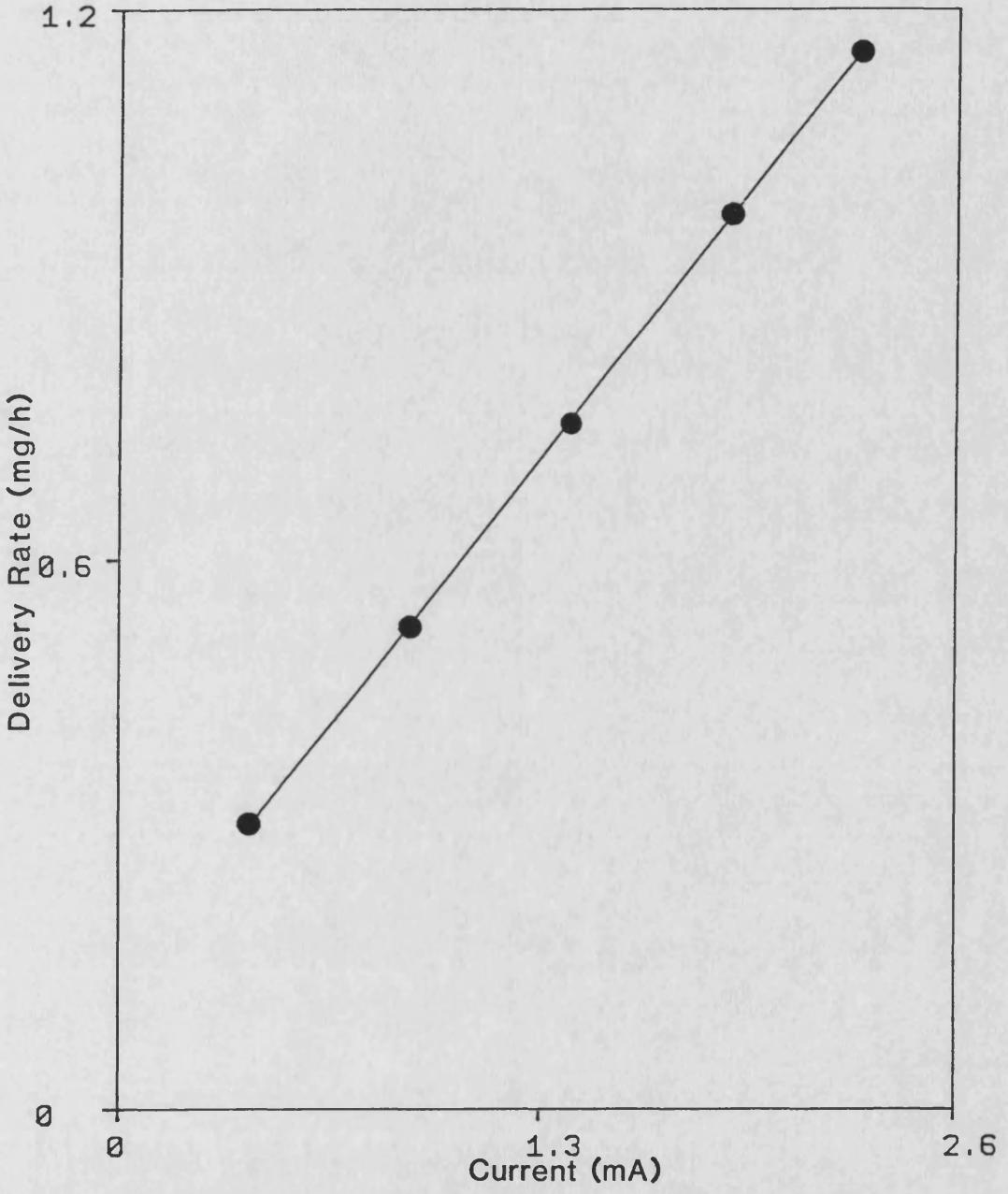


Figure 3.7. Effect of applied constant current on the transport of propranolol HCl through 1%C PHEMA films.

statistics :

Number of points	:	5
r^2	:	0.9999
Slope (S.D)	:	0.4448 (0.0024)
Intercept (S.D)	:	0.1297 (0.0037)

The intercept of the line in figure 3.7 with the y-axis represents the approximate delivery rate of propranolol HCl under diffusion control, without any electrophoretic contribution. Previous studies on electrophoretic drug delivery devices have shown a similar linear relationship when insulin was investigated using constant voltages [127] and bovine serum albumin using constant currents [128], both these studies used polyacrylamide as the membrane material.

An interesting observation was that the delivery rate of propranolol HCl due to diffusion prior to electrophoresis varied slightly in each experiment due to slight differences in film thickness and initial reservoir concentration. These differences however were not reflected in changes in delivery rate produced during electrophoresis, the delivery rate for any given current showing very little variability between the replicates.

On cessation of electrophoresis, the drug delivery rate decreased significantly in less than 20 minutes and quickly approached the pre-electrophoresis level (see figure 3.3 and appendix 4). However, the instantaneous value of the post-electrophoresis delivery rate was found to be up to 30% greater than the delivery rate pre-electrophoresis, although this delivery rate usually decreased down to a value within 5% after about 6 to 10 hours from the cessation of electrophoresis. A possible explanation for this

effect is that during electrophoresis the PHEMA disc is loaded with a higher level of propranolol HCl than would normally be present during diffusion, so that on removal of the current, this "extra" propranolol HCl is released before the concentration returns to its "normal" level.

The recorded changes in voltage that occurred during electrophoresis were similar in shape for all currents examined. A detailed study of the relationship between current and voltage is found in section 3.2.8. A typical relationship between measured voltage across the electrodes and time (figure 3.8) shows that the voltage rises rapidly and plateaus to give a near constant value during electrophoresis. It can therefore be assumed that electrophoresis was carried out under near constant power conditions. Different results were found when the changes in voltage during constant current polyacrylamide electrophoresis were examined [128]; an initial voltage was measured, which after a lag time of about one hour (corresponding to the lag time of bovine serum albumin release) reached a maximum value, then decayed slowly to a value obtained during the lag time so that electrophoresis was carried out under conditions of decreasing power. This effect may be due to the fact that diffusion of the bovine serum albumin had not attained steady state before electrophoresis was commenced (section 3.3.10).

The results obtained showed that constant current electrophoresis in the range 0 to 2.5 mA had a significant effect on the delivery rate of propranolol HCl through 1%C PHEMA films (figure 3.3, appendix 4). The effect is rapid and reversible. The results also indicated that the delivery rate of propranolol HCl during electrophoresis can be predicted using currents in the range examined

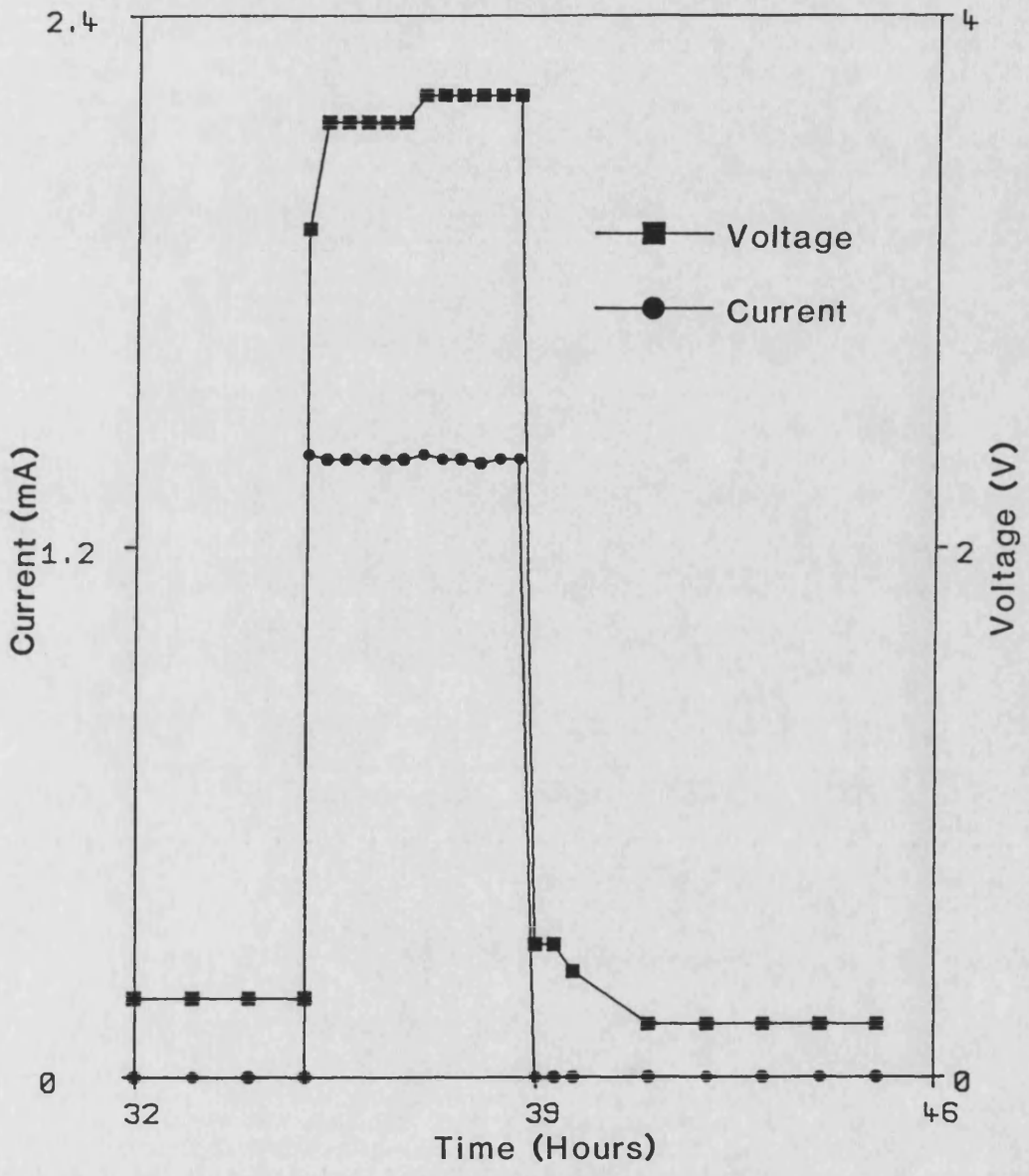


Figure 3.8. Changes in voltage measured during electrophoresis at a constant current of 1.4 mA.

(figure 3.7). The linear relationship between drug delivery rate and current probably extends beyond the range examined, however the limiting value of current that would be suitable for an electrophoretically controlled drug delivery device would be determined by degradation of drug and whether significant electrolysis of water occurred.

3.3.4 Effect of PHEMA Crosslinker Content on Electrophoresis

The effect of PHEMA crosslinker content on the delivery rate of propranolol HCl produced by constant current electrophoresis is shown in figure 3.9. Linear regression analysis of the three curves indicated that for 0.1%C PHEMA and 1.0%C the relationship between delivery rate and current is linear, however for 7.0%C the relationship appears slightly curvilinear. The results are surprising and do not follow the pattern of delivery which might be expected; in the current range examined the delivery rate produced for a given current was greatest for 1%C PHEMA followed by 0.1%C PHEMA and 7%C PHEMA. This was not expected because the diffusion coefficients, calculated from the steady state diffusion period prior to electrophoresis, showed the following expected order: 0.1%C PHEMA > 1.0%C PHEMA > 7%C PHEMA. The lines of best fit for the 0.1%C PHEMA and 1.0%C PHEMA, if extrapolated to zero current, cross over and give approximate intercepts of drug delivery produced by diffusion alone, with the intercept being greatest for the 0.1%C PHEMA film, as would be expected. Thus the change produced in drug delivery rate caused by changes in current are greater for the 1.0%C PHEMA than the 0.1%C PHEMA. The order of drug delivery produced by the various crosslinked polymers is also surprising when the

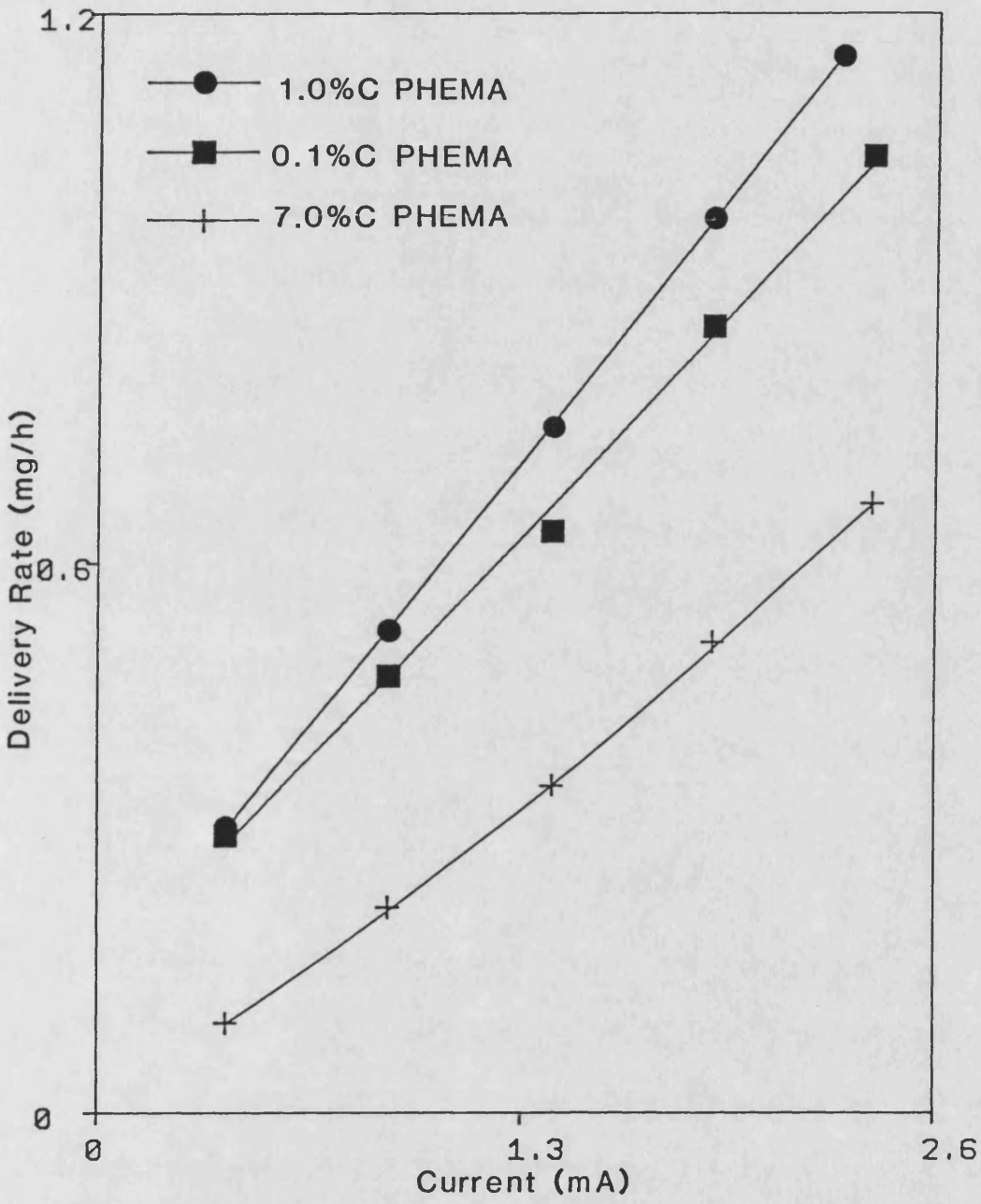


Figure 3.9. Effect of crosslinker content on the electrophoretic transport of propranolol HCl through PHEMA films.

electrical resistance across these films is measured (table 3.2); the pattern of resistance follows the expected order with 0.1%C PHEMA producing the lowest resistance. The curvilinear nature of the relationship between delivery rate and current with 7%C PHEMA is not easily interpreted, as the relationship between voltage and current in section 3.3.8 was linear, indicating that the resistance across the film was constant over the current range examined.

Previous electrophoretic studies using polyacrylamide have shown that as crosslinker content increases, electrophoretic mobility decreases [267,272,273]. Recent studies into electrophoretic drug delivery devices using polyacrylamide have however shown a biphasic relationship between mobility and crosslinker content; as crosslinker content is increased initially, a decrease in mobility is found, which reaches a minimum and then increases again as crosslinker content is increased further [127,128]. This effect was attributed to a re-arrangement of the polyacrylamide network with changes in crosslinker content. At low crosslinker contents, as crosslinker concentration is increased, a decrease in gel mesh size is thought to occur, and thus a decrease in mobility is found. At higher crosslinker contents a re-arrangement of the network is thought to occur resulting in an increase in mesh size and thus increase in mobility. A similar pattern of delivery rate and crosslinker content may be followed with PHEMA, however insufficient data is available to confirm this hypothesis. There is no evidence to suggest however that such a re-arrangement of PHEMA structure occurs as crosslinker content is altered.

An alternative explanation for the effect found in the present study is that the structure of PHEMA may somehow be affected by the

application of an electrophoretic field, indeed polyacrylamide has been shown to swell slightly during electrophoresis [127]. If there is a change in structure occurring as a result of electrophoresis, then this change may be related to the electric field strength, which is directly related to the applied electrophoretic current. In the case of both 0.1%C PHEMA and 1%C PHEMA, transport within PHEMA is believed to be via a "pore mechanism" (section 2.3.8), the diffusion coefficients of these two crosslinked films being similar (figure 2.12). One would expect a more rigid structure in the higher crosslinked 1.0%C PHEMA than the 0.1%C PHEMA film, thus the 1.0%C PHEMA may be more resistant to changes in structure produced by an electric field. This effect may explain the results obtained if the changes in the structure of PHEMA caused by an electric field results in the transport of propranolol HCl through PHEMA being hindered. This explanation however may be an oversimplification since changes in the crosslinker content in PHEMA has effects on both the effective pore size and the nature of water within the polymer (section 1.6.1).

The crosslinker content can also determine the type of effect produced by a constant current. Figure 3.10 shows the relationship between receptor propranolol HCl concentration and time using a 7%C PHEMA film, together with the effect of application of a current of 2.4 mA during experimentation. The effect is in contrast to that produced with 1%C PHEMA in figure 3.3. The ratio between delivery rate during electrophoresis and delivery rate due to diffusion prior to an electrophoretic current of 1.9 mA is 32 for 7%C PHEMA. The same ratios calculated for 0.1%C PHEMA and 1.0%C PHEMA are 6 and 7.5 respectively. Thus with the lower crosslinked PHEMA (0.1%C and 1.0%C), even though a much higher delivery rate is produced by a

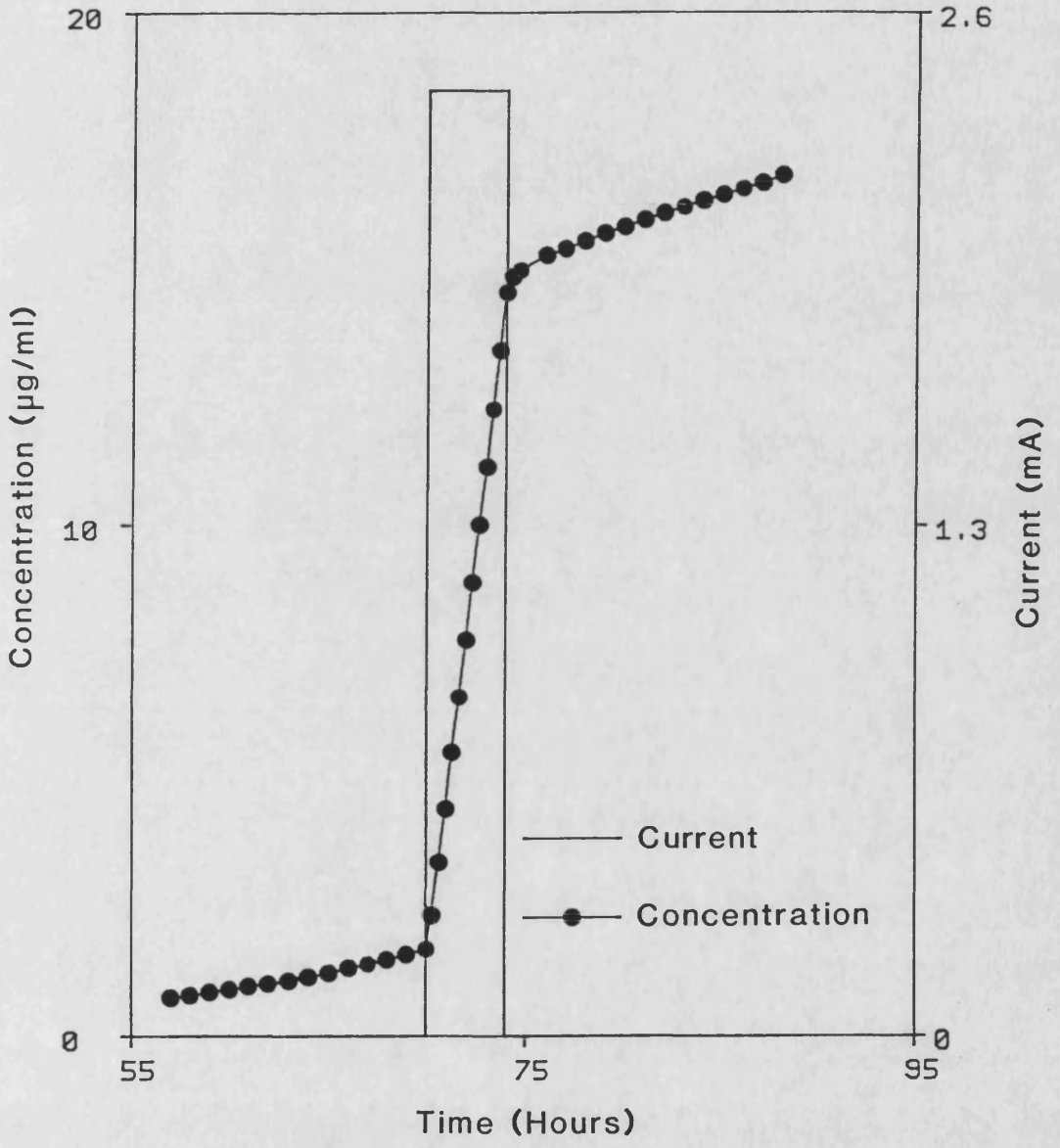


Figure 3.10. Effect of electrophoresis on the transport of propranolol HCl through 7%C PHEMA film.

given current, the change in delivery rate caused by electrophoresis over that of diffusion is much greater with the higher crosslinked 7%C PHEMA. It was reported in section 2.3.8 that a change in diffusion mechanism is thought to occur as the crosslinker content in PHEMA is increased, and it would therefore be expected that transport of propranolol HCl through 0.1%C PHEMA and 1.0%C PHEMA would be by a different mechanism to that of 7%C PHEMA. This difference in mechanism may be the cause of the different effects produced by the highly crosslinked 7%C PHEMA and the lower crosslinked PHEMA films. A difference in the properties of the films is also indicated by the resistance data (section 3.3.8); the resistance across the 0.1%C and 1.0%C films were found to be very similar where as the resistance across 7%C PHEMA was found to be much greater.

3.3.5 Effect of Buffer Ionic Strength on Electrophoresis and pH Changes During Electrophoresis

The measured pH's for the acetate buffers of various ionic strengths were found to be in close agreement with the theoretically predicted values as shown below :

Ionic Strength	Theoretical pH	Measured pH
0.039	4.481	4.484
0.080	4.457	4.439
0.120	4.442	4.406
0.160	4.432	4.387
0.200	4.424	4.381

The u.v. scans of the 0.039 and 0.20 ionic strength buffers showed no significant differences, therefore the u.v. assay of

propranolol HCl used in electrophoresis experiments was considered to be unaffected by the presence of sodium chloride.

The effect of ionic strength on the electrophoretic delivery of propranolol HCl is shown in figure 3.11. As can be seen, at a constant current of 2.4 mA, as ionic strength is increased, the delivery rate decreases, tending towards a value for delivery rate which is produced by diffusion alone. Figure 3.12 shows the effect of ionic strength on pre-electrophoresis drug delivery due to diffusion, the results indicate that an increase in ionic strength causes a slight decrease in the delivery rate produced by diffusion alone, this decrease is probably due to a reduction in the effective reservoir concentration of propranolol HCl due to activity effects [132]. A similar effect of ionic strength on electrophoresis has recently been reported on the delivery rate of bovine serum albumin produced by a constant electrophoretic current [128]. The effect of ionic strength on the delivery rate can be explained by two main effects that are occurring as ionic strength is changed. The first and least significant effect in this particular model, occurs as a result of the changes in the conductance of propranolol cations arising from electrophoretic and relaxation phenomena, which are the result of concentration dependent interactions between ions in solution (section 1.4.1). The Onsager equation relates ionic strength to the changes in conductance that result from these effects. A

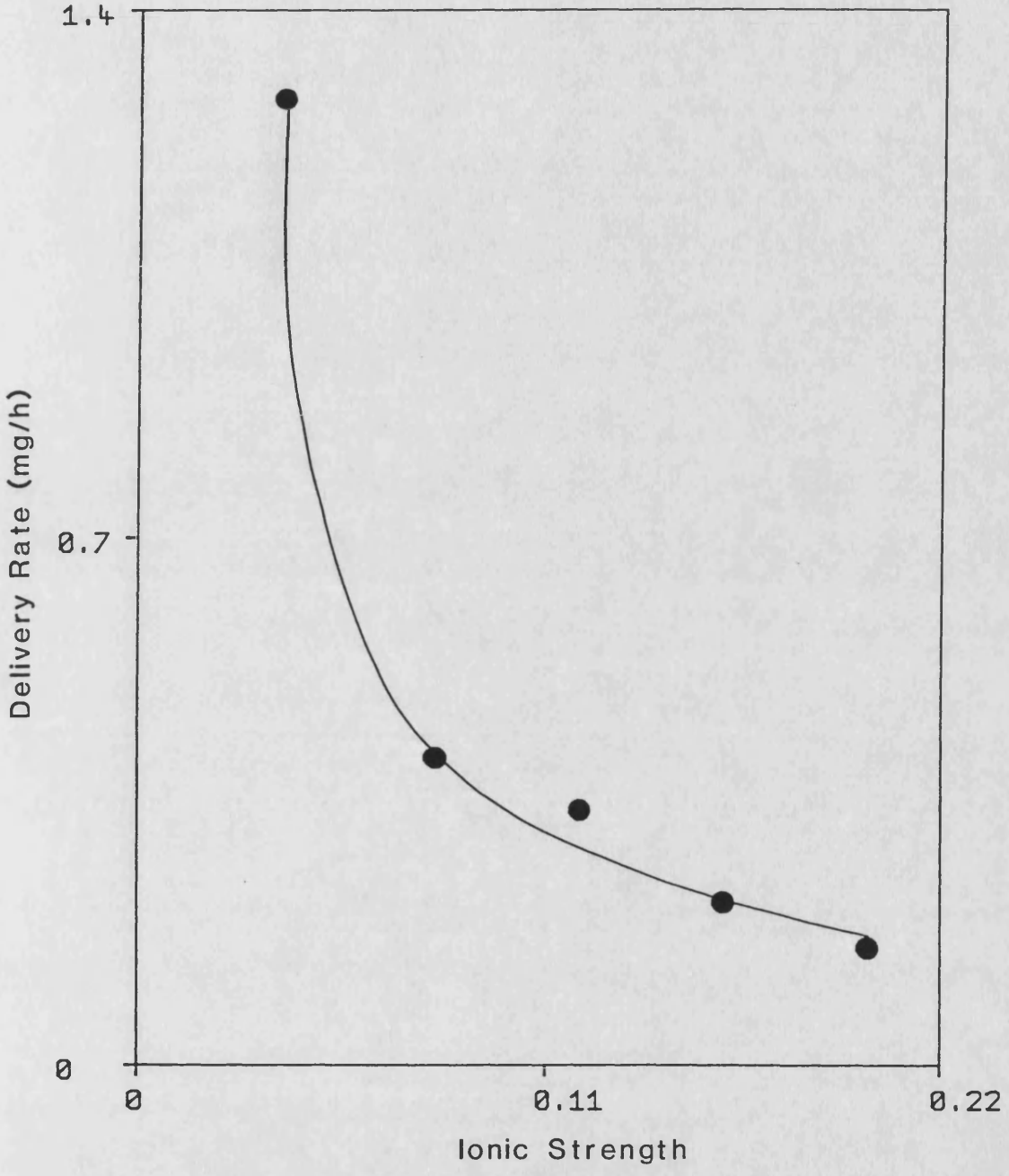


Figure 3.11. Effect of ionic strength on the electrophoretic transport of propranolol HCl through 1%C PHEMA film at a constant current of 2.4 mA.

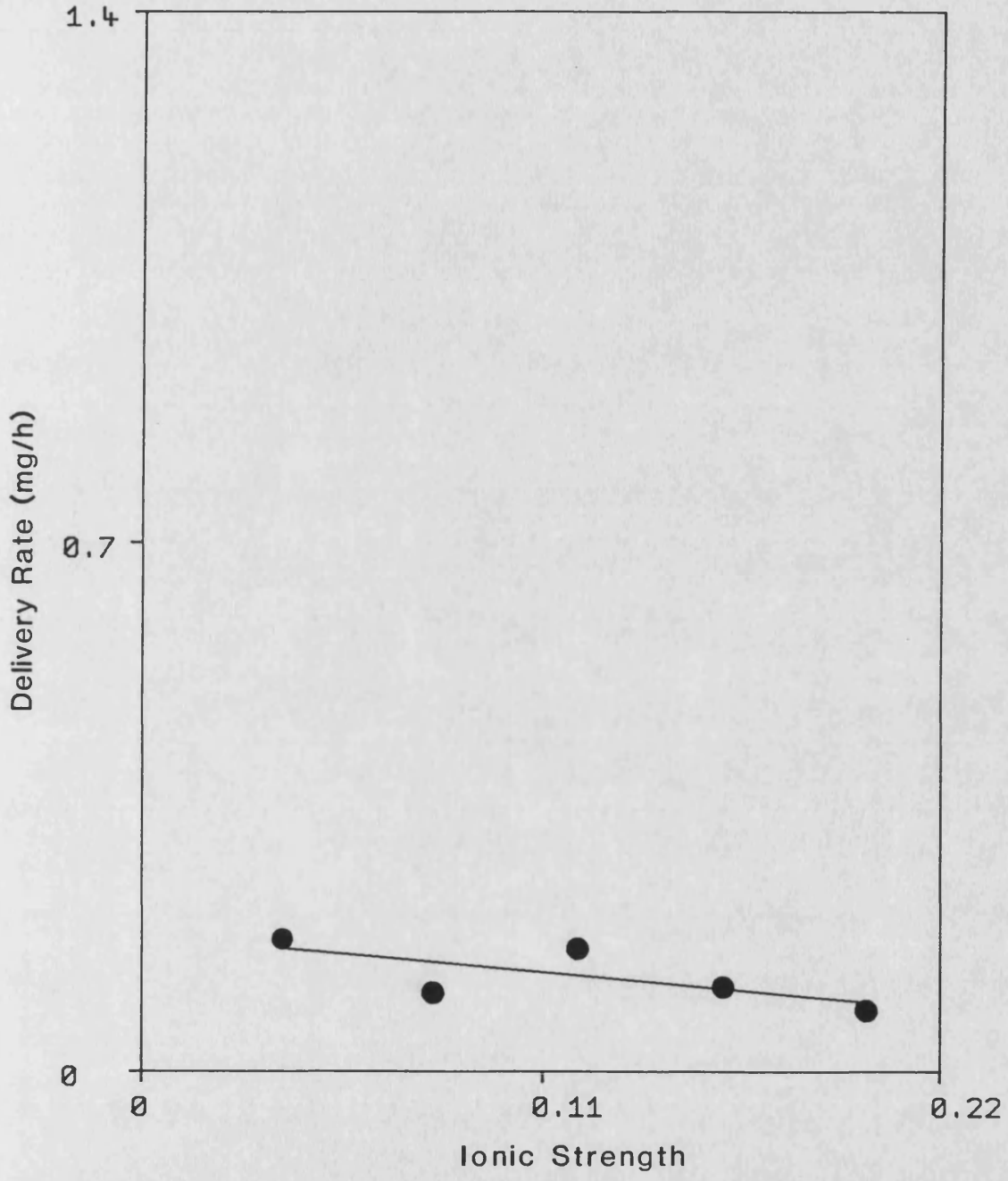


Figure 3.12. Effect of ionic strength on the pre-electrophoretic transport (due to diffusion) of propranolol HCl through 1%C PHEMA film.

simplified form of the relationship is :

$$\Lambda = \Lambda_o - (A + B\Lambda_o).I^{1/2}$$

Λ = Molar Conductivity (Ω^{-1} m^2 mol^{-1})

Λ_o = Molar Conductivity at Infinite Dilution (Ω^{-1} m^2 mol^{-1})

I = Ionic Strength

A = Constant

B = Constant

Molar conductivity is the conductivity of an electrolyte divided by its molar concentration, thus an inverse relationship between increasing ionic strength and molar conductivity exists. This relationship is significant in the present work because it shows that for a fixed concentration of propranolol HCl an increase in ionic strength would be expected to decrease the conductivity of propranolol HCl.

The second and more significant reason for the decrease in drug delivery rate is related to the concentration of ions in solution and that electrophoresis of propranolol HCl was carried out in constant current conditions. Electric current is a measure of the rate at which electric charge is transported, and thus the rate at which ions are transported into the receptor [134]. Since in this study the effect of constant current was being investigated, irrespective of the ionic strength of the buffer, the same number of ions will be transported over a given period. As ionic strength is increased, the proportion of propranolol cations to the total number of ions in solution decreases, thus one would expect the amount of propranolol HCl transported during electrophoresis to decrease as ionic strength increases. Gross [274] predicted that electrophoretic mobility should

be inversely proportional to the square root of ionic strength; a plot of the ratio of drug delivery rate to electrical resistance against the reciprocal of the square root of ionic strength is shown in figure 3.13. Since a constant current was used in this study, the electric field strength will be proportional to the measured resistance (section 3.3.8), thus the electrophoretic mobility will be proportional to the ratio of drug delivery rate to resistance. The results from this study indicate that electrophoretic mobility is inversely proportional to the square root of ionic strength as predicted by Gross [274]. It should be noted that in this study the contribution of propranolol HCl to the ionic strength was not considered, and that a slightly higher ionic strength prevailed in the reservoir than the receptor compartment due to the higher concentration of propranolol HCl.

The resistance across the electrodes was found to decrease as ionic strength was increased due to an increase in conductivity of the buffer system. It was observed that at ionic strengths of 0.12 and above, at the end of electrophoresis the anode had a fine mauve coloured film, which increased in colour intensity as the ionic strength was increased. It was found that the film was easily removed with concentrated sulphuric acid, the resulting solution having a green colouration. It was considered that the film may have been due to an accumulation of chloride ions since the same effect occurred at high reservoir concentrations of propranolol HCl (section 3.3.7) although the nature of the electrode reaction is not understood.

The results indicated that ionic strength had a significant effect on the electrophoresis of propranolol HCl, higher ionic strengths decreasing the delivery rate produced by a given

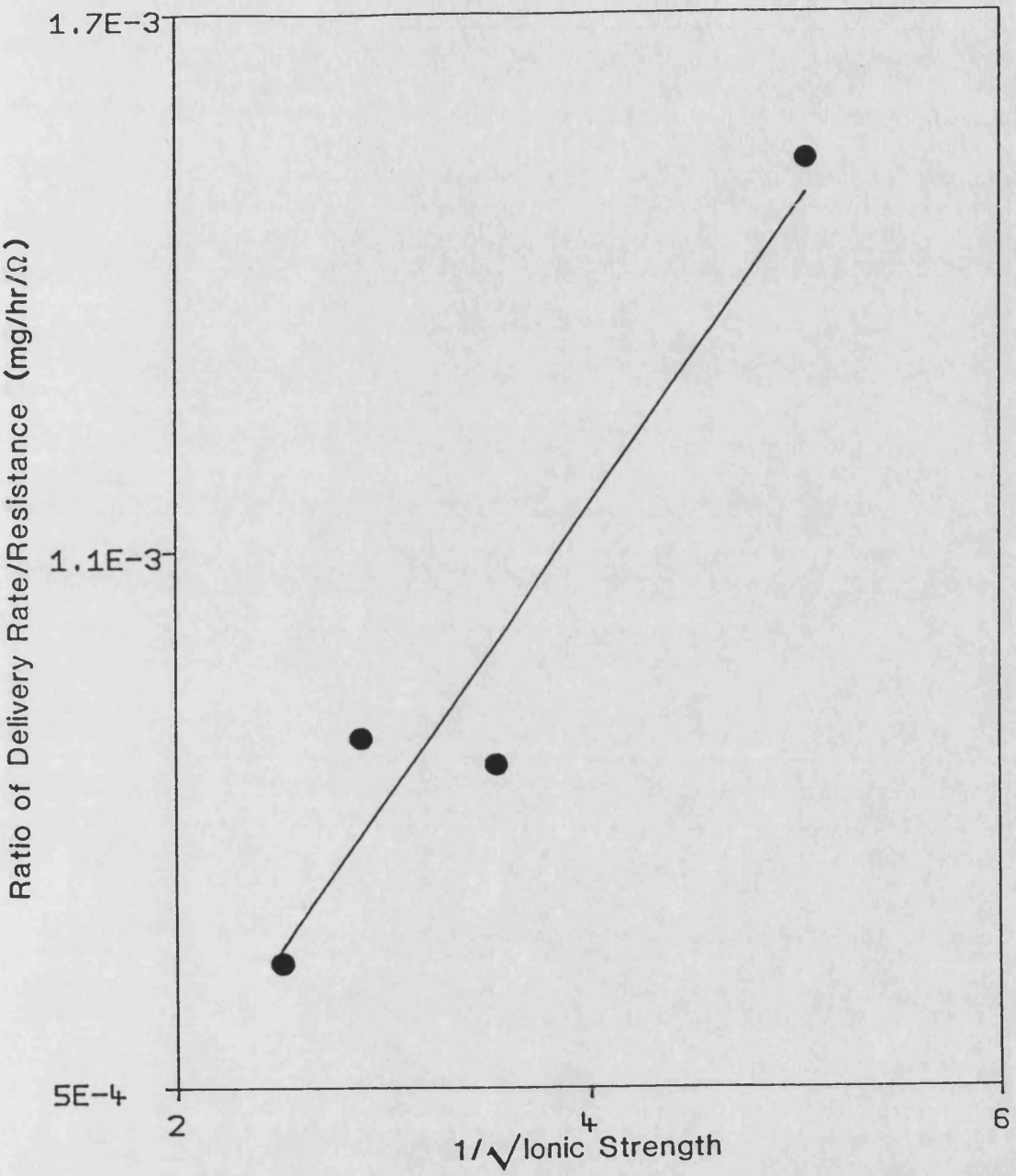


Figure 3.13. A plot of the ratio of drug delivery rate to electrical resistance versus the reciprocal of the square root of ionic strength.

electrophoretic current. This may prove to be a limitation on the use of an electrophoretic device for drug delivery in a varying or high ionic strength environment. In order to investigate further it would be interesting to examine the effect on electrophoresis of having a relatively low ionic strength in the reservoir compartment and high ionic strength in the receptor compartment (the environment of the device) and examining whether changes in the ionic strength of the receptor compartment alone have a significant effect on electrophoretic drug delivery.

The changes in pH that occurred during the study of ionic strength effects on drug delivery rates are shown in table 3.1. The values shown are the difference between pH after electrophoresis and pH before electrophoresis. The results show that as a result of electrophoresis there is a slight decrease in pH in the reservoir (anolytic) compartment and a slight increase in pH in the receptor (catholytic) compartment. These changes may be the result of low level electrolysis of water, although no evolution of gases was seen at the electrodes, possibly because the rate of gas production was too low to allow visible build up. The electrolysis of water may be represented as follows [275] :

Anode $\quad 6H_2O \longrightarrow O_2 + 4H_3O^+ + 4e^-$

Cathode $\quad 4H_2O + 4e^- \longrightarrow 2H_2 + 4OH^-$

Ionic strength did not appear to have any significant effect on the changes in pH.

Table 3.1 The relationship between changes in pH and ionic strength found after electrophoresis of propranolol HCl in acetate buffer carried out for four hours at 2.4 mA.

Ionic Strength	Reservoir Compartment pH	Receptor Compartment pH
0.039	-0.026	0
0.080	-0.038	+0.013
0.120	-0.031	+0.003
0.160	-0.035	+0.023
0.200	-0.051	+0.007

3.3.6 Effect of Temperature on Electrophoresis

The effect of temperature on the electrophoretic transport of propranolol HCl through films of 1%C PHEMA was investigated. Figure 3.14 shows the effect of current on the electrophoretic delivery of propranolol HCl through 1%C PHEMA at different temperatures, figure 3.15 shows the relationship between delivery rate and temperature at different currents. For any given current it can be seen that as temperature is increased so does the drug delivery rate. In addition the rate of change of delivery rate with current increases as temperature is increased. Figure 3.16 shows the relationship between the logarithm of the delivery rate per unit current and the reciprocal of absolute temperature, the results suggest that the effect of temperature on the relationship between drug delivery rate and current may be described by the Arrhenius equation [134]. The effect of increasing temperature may be explained by an associated increase in free diffusion and ionic mobility, together with a decrease in the viscosity of the electrophoresis medium [267]. The

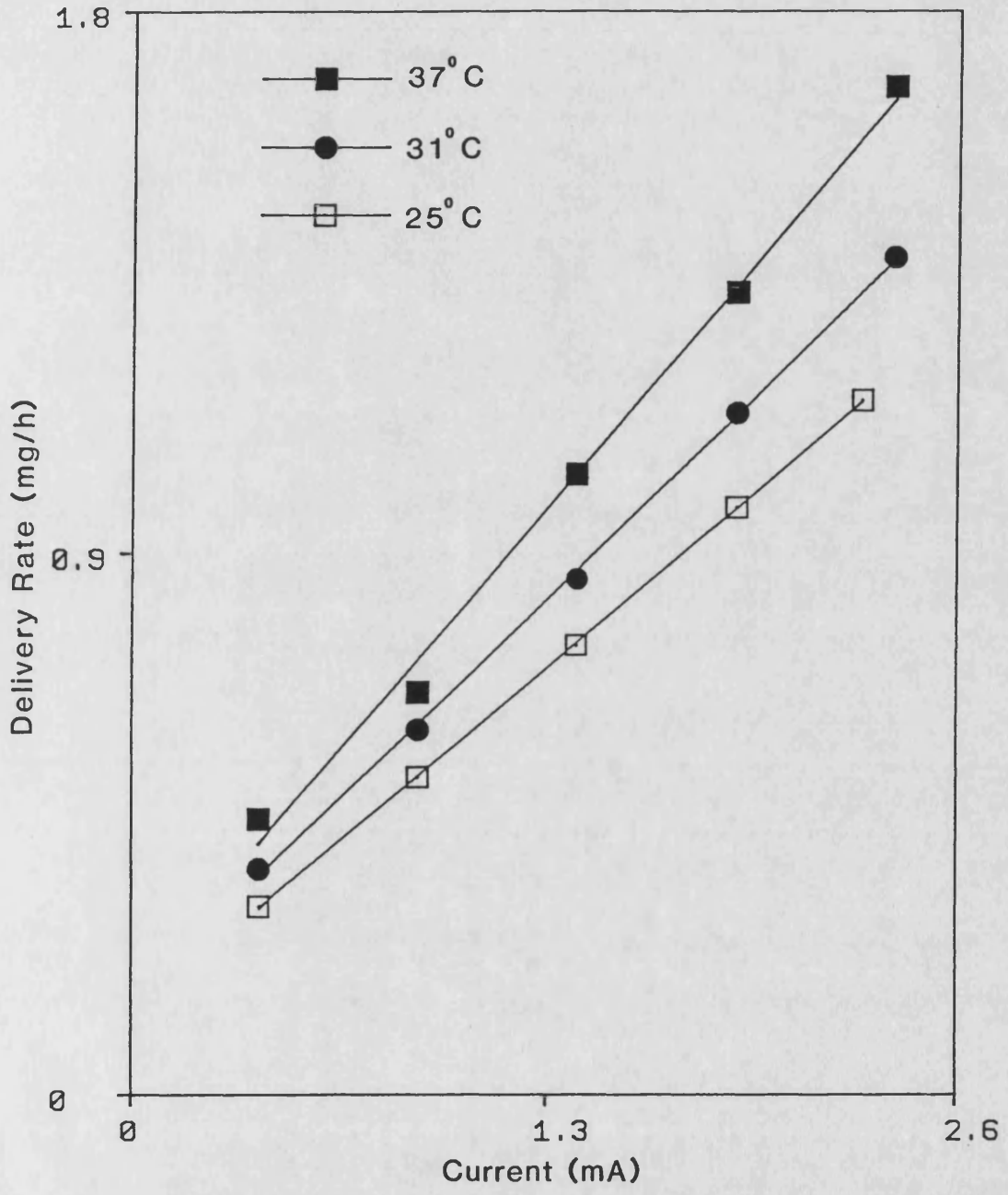


Figure 3.14. Effect of current on the electrophoretic delivery of propranolol HCl through 1%C PHEMA films at different temperatures.

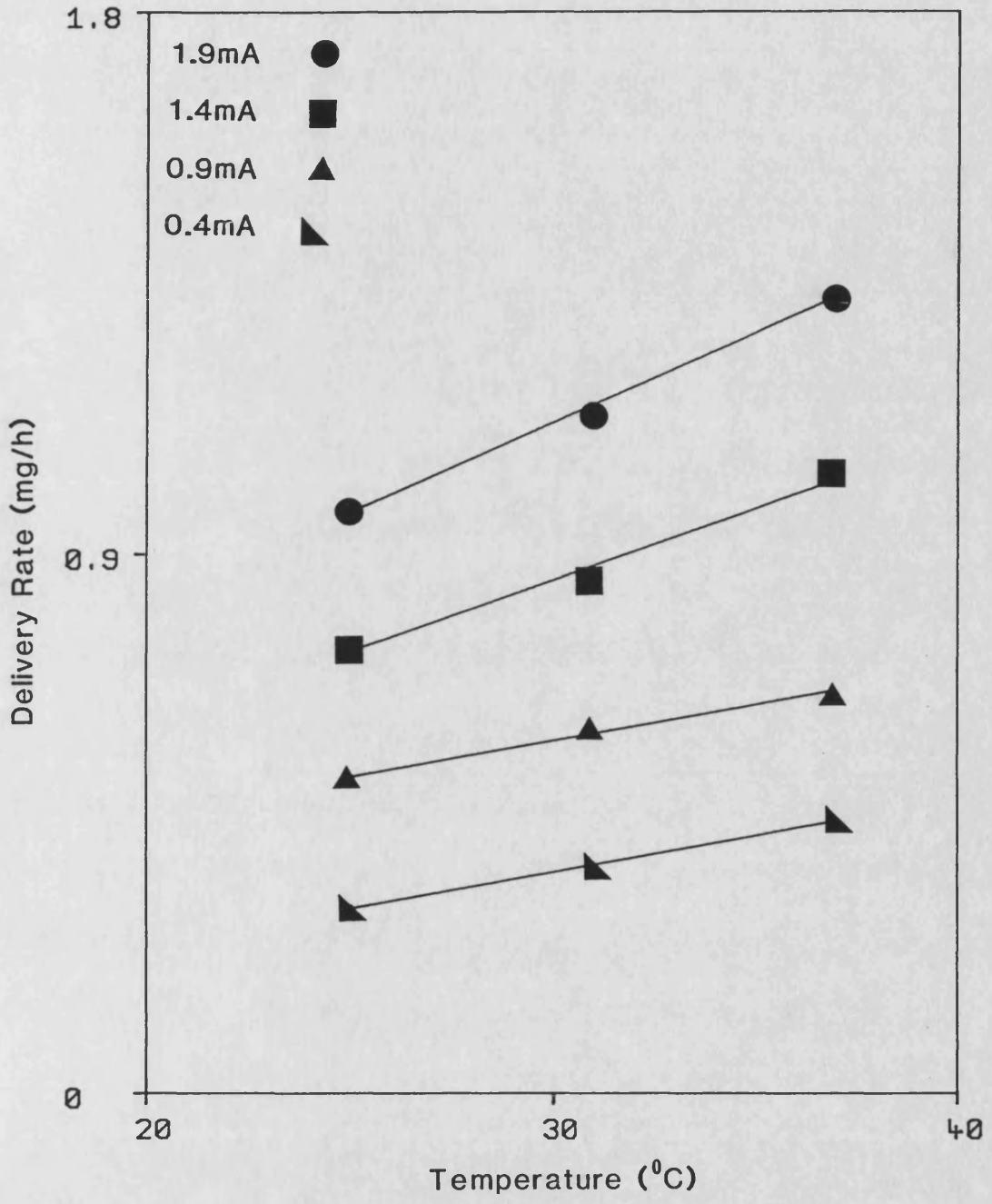


Figure 3.15. Effect of temperature on the electrophoretic delivery of propranolol HCl through 1%C PHEMA films at different constant currents.

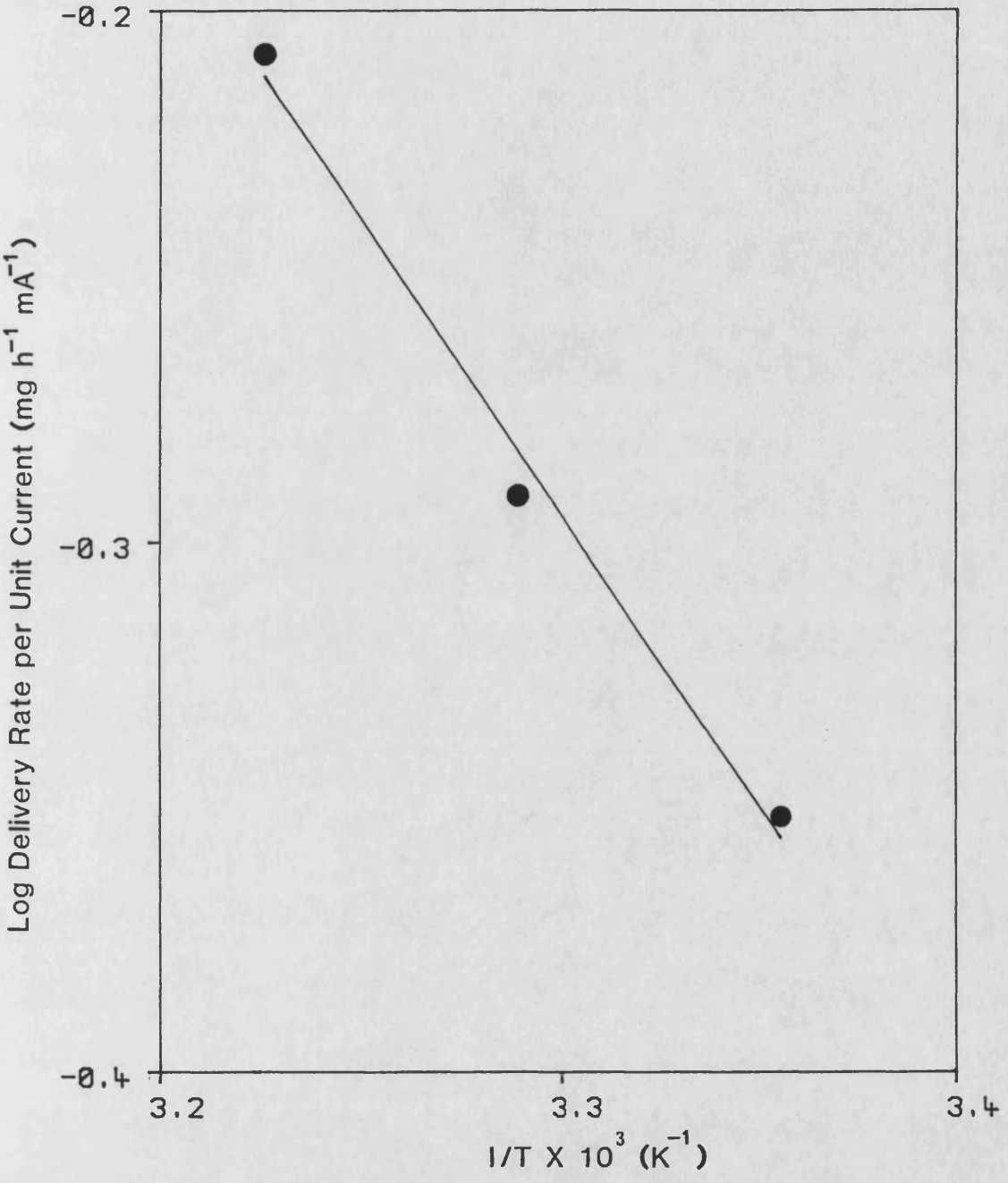


Figure 3.16. A plot of log delivery rate per unit current versus the reciprocal of absolute temperature.

effect of temperature on the electrical resistance across the 1%C PHEMA films was also determined (section 3.3.8) and it is interesting to note that a linear relationship exists between the logarithm of electrical resistance and the reciprocal of absolute temperature (figure 3.17). An analogy may be made with the viscosity of liquids where an equation analogous to the Arrhenius equation is also found to describe the dependence of viscosity on temperature [136].

3.3.7 Effect of Reservoir Propranolol HCl Concentration on Electrophoresis

The effect of varying the reservoir concentration of propranolol HCl on the delivery rate produced by an electrophoretic current of 1 mA was studied. Figure 3.18 shows that as reservoir concentration was increased initially, a rapid increase in drug delivery rate due to electrophoresis occurred. However, the gradient of the delivery rate versus concentration curve became less steep as reservoir concentration increased. It was also found that as the delivery rate due to electrophoresis increased, so did the measured delivery rate due to diffusion prior to electrophoresis (figure 3.19). Figure 3.19 also shows that as the concentration of reservoir propranolol HCl increased, the change in drug delivery rate produced by an electrophoretic current of 1 mA decreased, tending towards a limiting value corresponding to that produced by diffusion alone. These results seem to indicate that the choice of reservoir concentration is critical: too high a concentration making electrophoretic control ineffective; too low a concentration being unsuitable for practical use as a drug reservoir. The influence of concentration on the effect produced by constant voltage electrophoresis was found to be complex

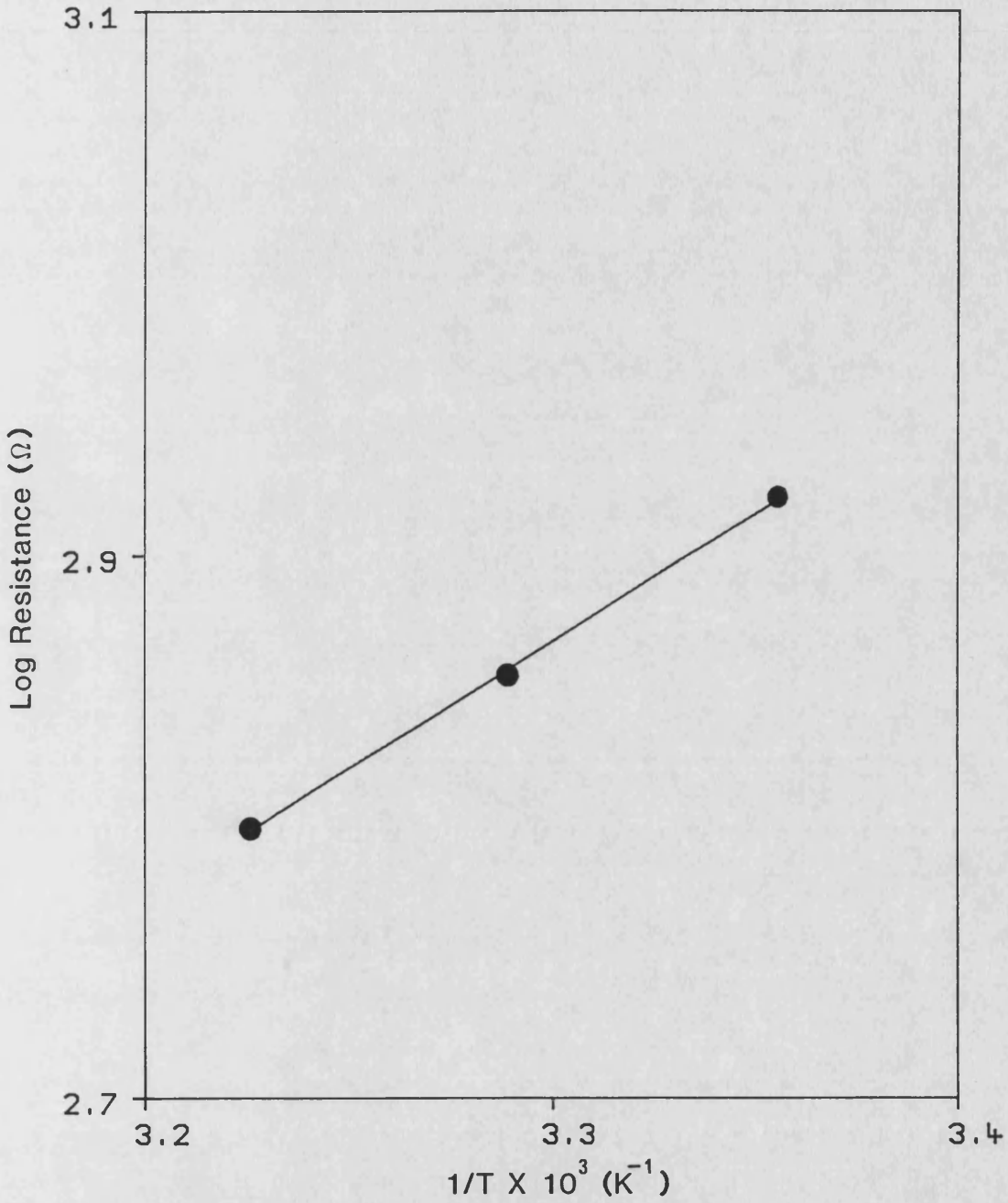


Figure 3.17. A plot of log resistance versus the reciprocal of absolute temperature.

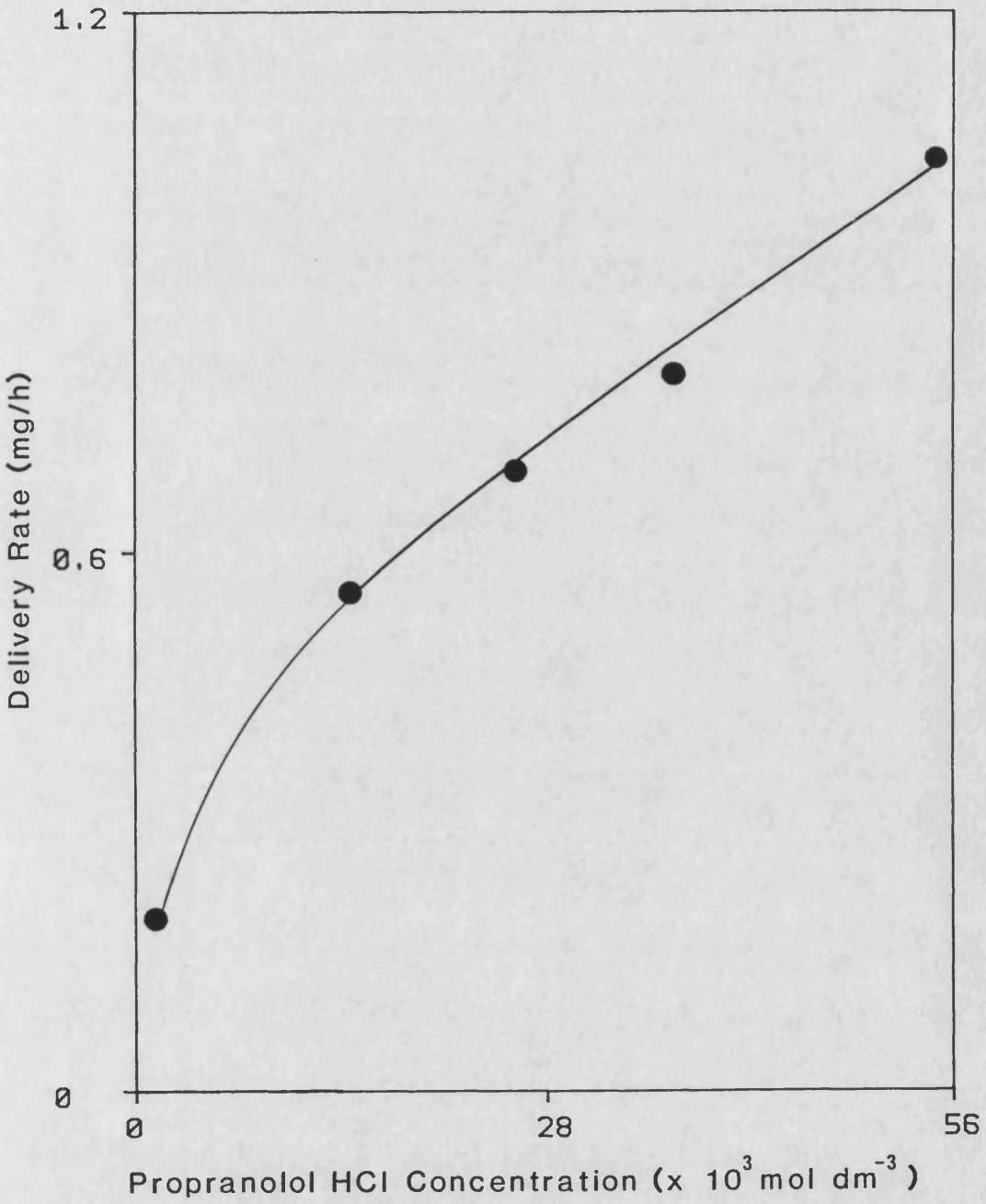


Figure 3.18. Effect of propranolol HCl reservoir concentration on the electrophoretic delivery of propranolol HCl through 1%C PHEMA films at a constant current of 1 mA.

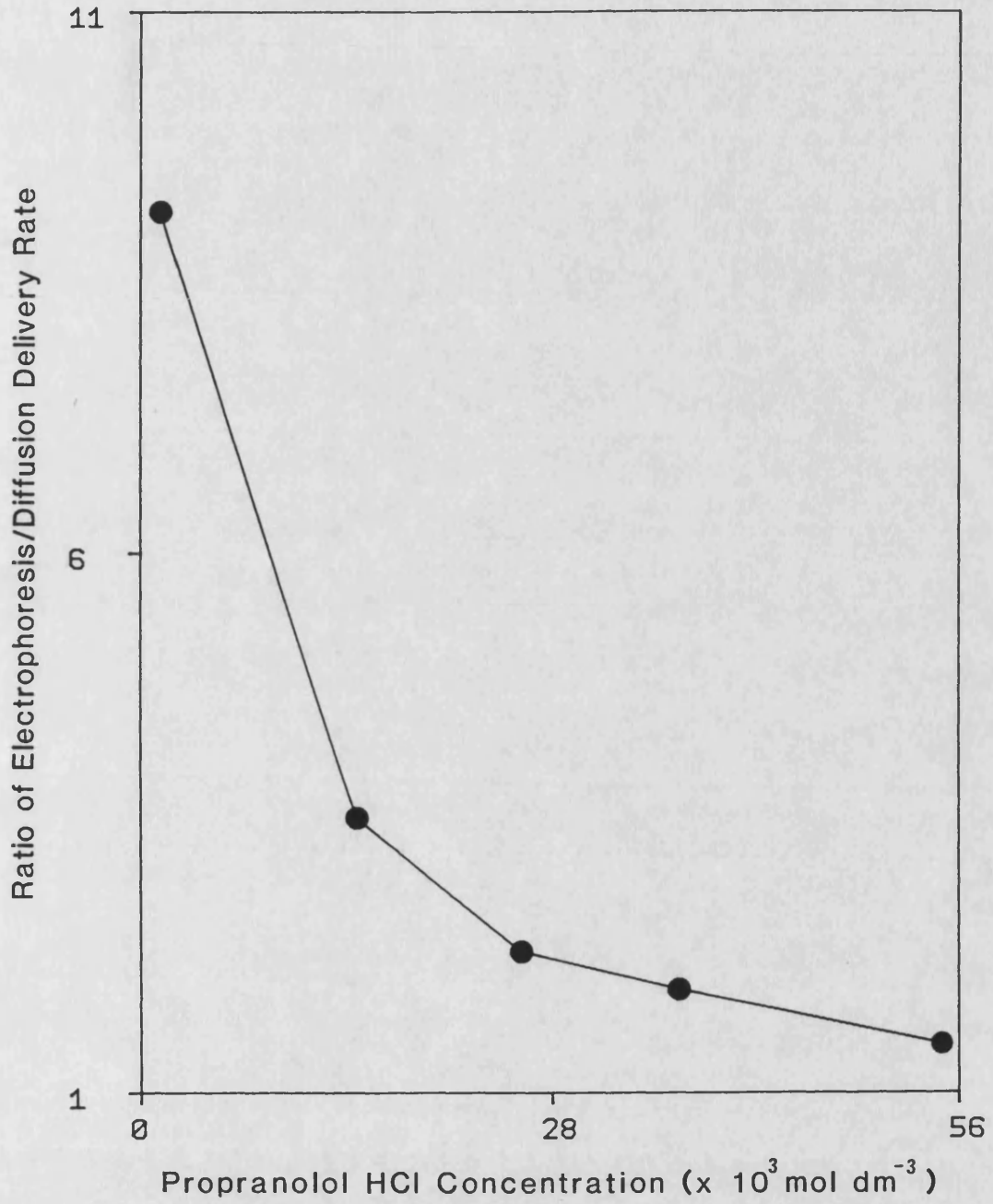


Figure 3.19. Effect of propranolol HCl reservoir concentration on the change in delivery rate produced by an electrophoretic current of 1 mA.

[127], however this may have been due to the fact that experiments were performed during the lag period of diffusion, as described earlier in section 3.3.3. The actual mechanism by which concentration affects drug delivery rate appears complex. As well as increasing the contribution of diffusion, increasing the reservoir concentration also increases the ionic strength of the reservoir compartment, and will affect the conductance of the propranolol cation as described in section 3.3.5.

It was observed that following electrophoresis using a propranolol HCl concentration of 54.9 mM, the anode had a fine mauve film, as found with high ionic strength buffer (section 3.3.5). As before, the film was easily removed with concentrated sulphuric acid, the nature of this film was associated with the accumulation of chloride ions (section 3.3.5).

3.3.8 Factors Affecting the Resistance Across PHEMA Films During Electrophoresis

Figure 3.20 shows the relationship between voltage and current across 1% PHEMA at 25°C with a buffer ionic strength of 0.039. The linear relationship between voltage and current in figure 3.20 was typical of that found in all experiments in this study, which allowed the resistance across the system to be calculated from the gradients of these plots. Linear regression was performed on all of the plots to check for linearity. The calculated resistance values are shown in tables 3.2 to 3.4. The results will be discussed further in context with other relevant sections.

It should be noted that plots of voltage versus current had significant positive intercepts with the the y-axis, ranging from

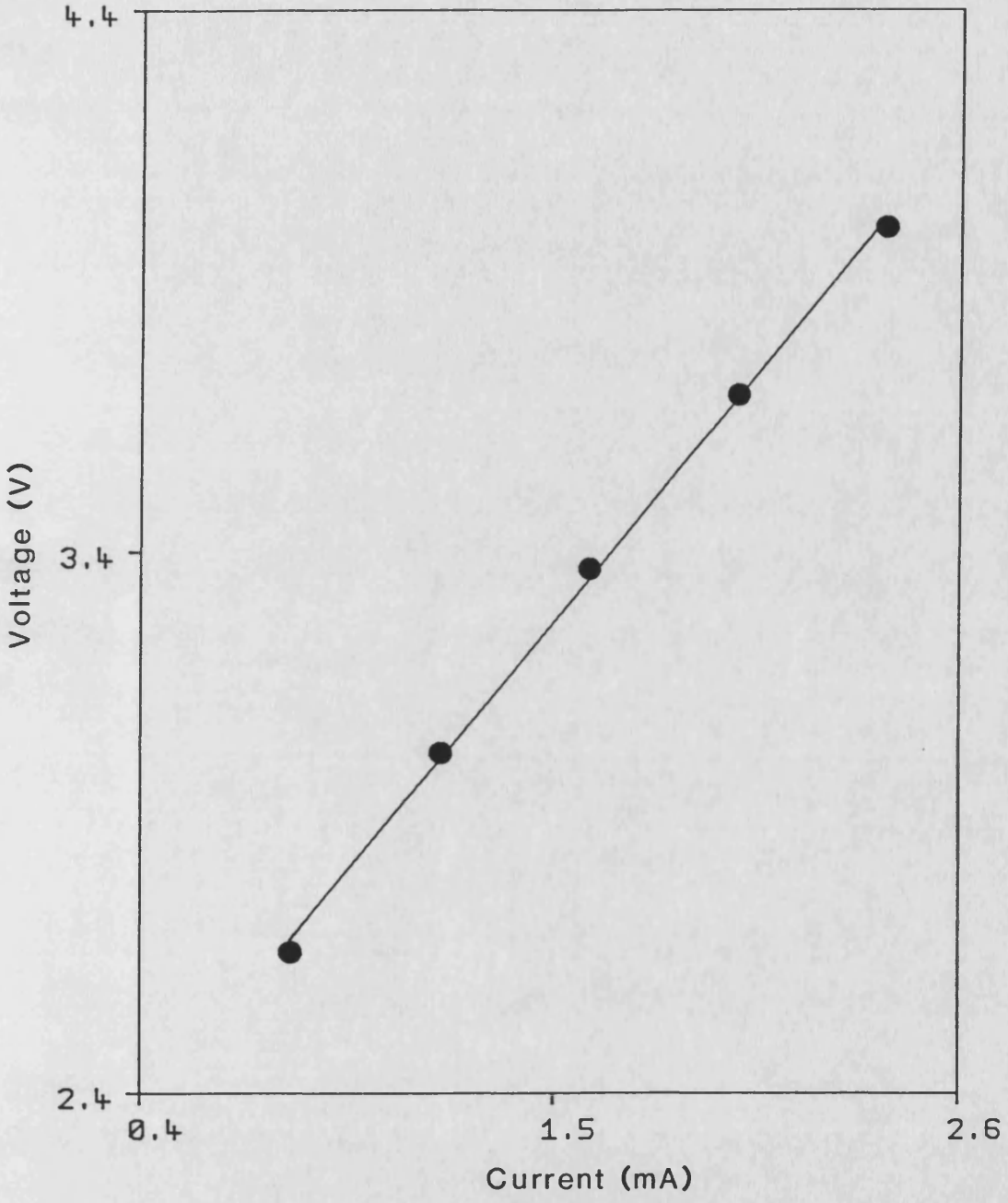


Figure 3.20. Relationship between voltage and current across 1%C PHEMA film.

Table 3.2 The effect of PHEMA crosslinker content on the measured resistance between the platinum electrodes at 25°C and an ionic strength of 0.039.

Crosslinker Content (%C)	Resistance (Ω)
0.1	778
1.0	835
7.0	1685

Table 3.3 The effect of temperature on the measured resistance between the platinum electrodes with 1%C PHEMA and an ionic strength of 0.039.

Temperature (°C)	Resistance (Ω)
25	835
31	718
37	630

Table 3.4 The effect of ionic strength on the measured resistance between the platinum electrodes with 1%C PHEMA at 25°C.

Ionic Strength	Resistance (Ω)
0.039	835
0.080	473
0.120	380
0.160	335

2.01 to 2.3 volts depending on the experimental conditions. This positive intercept may be explained by electrode polarization. The change in electrode potential resulting from polarization is termed the overpotential (overvoltage), and no current will flow between the electrodes unless the applied voltage exceeds the overpotential [276]. The appearance and magnitude of electrode polarization may be dependent on a number of factors including electrolyte concentrations, temperature, stirring rate and current density.

The values of resistance give an indication of the power requirements of an electrophoretic drug delivery device. If we consider the resistance of the model system at 37°C with 1%C PHEMA and an ionic strength of 0.039, then the power requirements can be calculated from the following relationship :

$$P = I^2.R$$

P = Power (W)

I = Current (A)

R = Resistance (Ω)

The calculated power requirements at a constant current of 2.0 mA would be 2.5 mW. These values have significant implications in respect of the use of a low-power electrophoresis drug delivery device for clinical use. Such a device could be powered by batteries, for example a typical button-type lithium cell is capable of supplying 200 mAh at a constant 3 V (RS Components Ltd., Corby, U.K.), which is approximately equivalent to 200 operating hours at the power consumption calculated above. Alternatively power for an electrophoretic device could be generated from transducers utilising the bodies own energy sources; for example a piezo-electric device could generate a current from the pulsing of a major artery, or

thermocouples could generate a current from the bodies own heat [277].

3.3.9 Effect of Reversing Electrode Polarity

Figure 3.21 shows the effect of changing the magnitude of the constant current on the electrophoretic transport of propranolol HCl through 1%C PHEMA, with the receptor electrode as the anode. It was found that as the current magnitude was increased from 0.7 mA to 1.2 mA, the delivery rate of propranolol HCl during electrophoresis decreased gradually, the delivery produced by a current of 0.9 mA was found anomalous, being lower than expected from subsequent higher electrophoretic currents. From the initial results a current was estimated (1.09 mA) which would give a zero delivery rate, and was applied for two successive periods. The results in figure 3.21 show that on the first application of a current of 1.09 mA, the delivery rate is practically zero, however on the second application the delivery rate actually becomes slightly negative in magnitude with the same current. This may be because the electrophoretic current is depleting the PHEMA film of propranolol HCl, thus reducing the contribution to delivery rate due to diffusion. Since the net delivery rate during electrophoresis is the sum of diffusional and electrophoretic effects, then a decrease in the diffusional component might be expected to produce a decrease in the delivery rate into the receptor compartment. This assumption may be valid since the measured delivery rates during the periods when no electrophoretic current is applied show a gradual decrease with time, the value being 0.148 mg/h at 30 hours, decreasing gradually to a value of 0.129 mg/h after 130 hours.

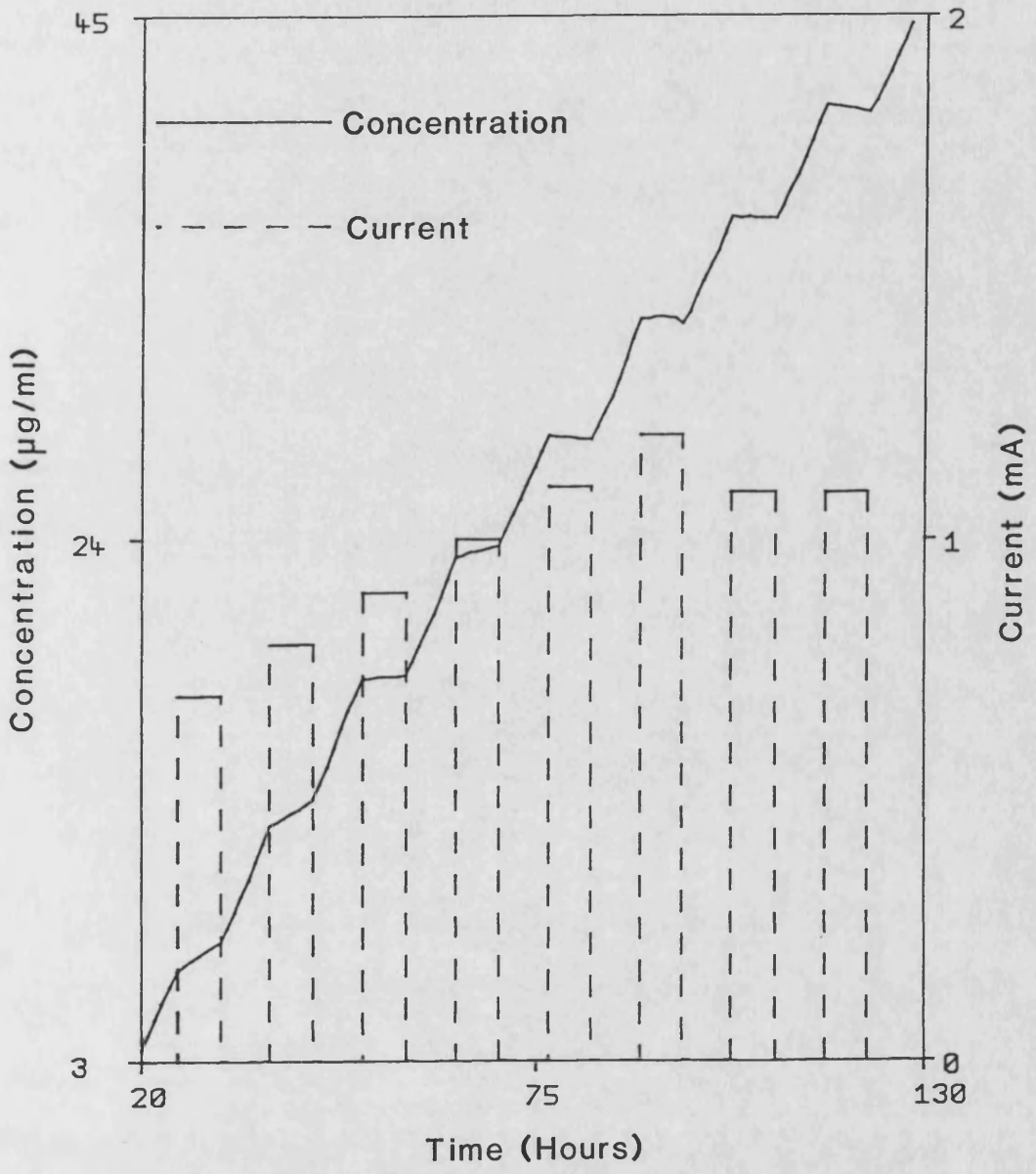


Figure 3.21. Effect of current on the transport of propranolol HCl through 1%C PHEMA film with the receptor electrode as the anode.

The results show that as well as being able to increase the delivery rate of propranolol HCl, constant current electrophoresis can also be used to reduce the delivery rate below the basal delivery rate produced by diffusion. Further, the results show that control of both the magnitude and polarity of the electrophoretic driving force allows drug delivery rates to be modulated continuously from zero up to the maximum limiting value.

3.3.10 Effect of Electrophoresis on Diffusion Lag Time

Figure 3.22 shows the relationship between propranolol HCl concentration in the receptor compartment and electrophoresis time. The results show a lag time of approximately 1.2 hours before a change in drug concentration occurred, which was much greater than the time taken for an electrophoretic current to produce a change in delivery rate during steady state diffusion (section 3.3.3). After the lag time, zero-order delivery of propranolol HCl was found to occur at a rate of 0.75 mg/h. This value is much less than that which would be expected if electrophoresis at 2 mA was performed after steady state diffusion had been attained, the expected value would be in the region of 1.02 mg/h (section 3.3.3). The voltage profile obtained against time was also markedly different to that obtained when electrophoresis was effected during steady state diffusion. Figure 3.8 shows a typical voltage-time profile for electrophoresis when the delivery rate due to diffusion is constant, figure 3.23 shows the profile obtained in the present study, the voltage reaching a peak corresponding to the lag time for drug appearance, the voltage then decreased gradually. The measured voltages in this study are also higher than would be expected if electrophoresis had been

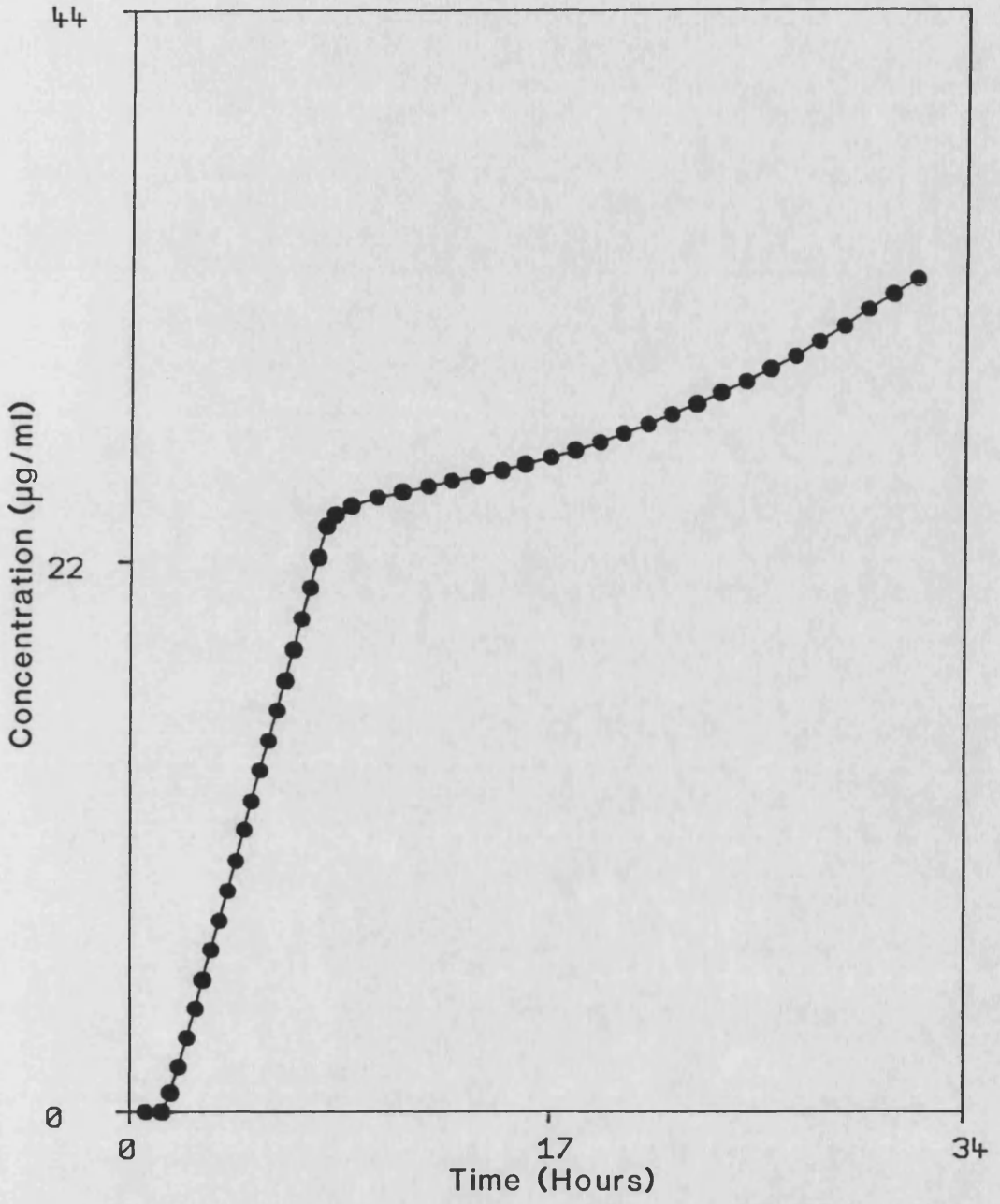


Figure 3.22. Effect of application of a constant electrophoretic current of 2 mA on the transport of propranolol HCl through 1%C PHEMA film during the diffusion lag time.

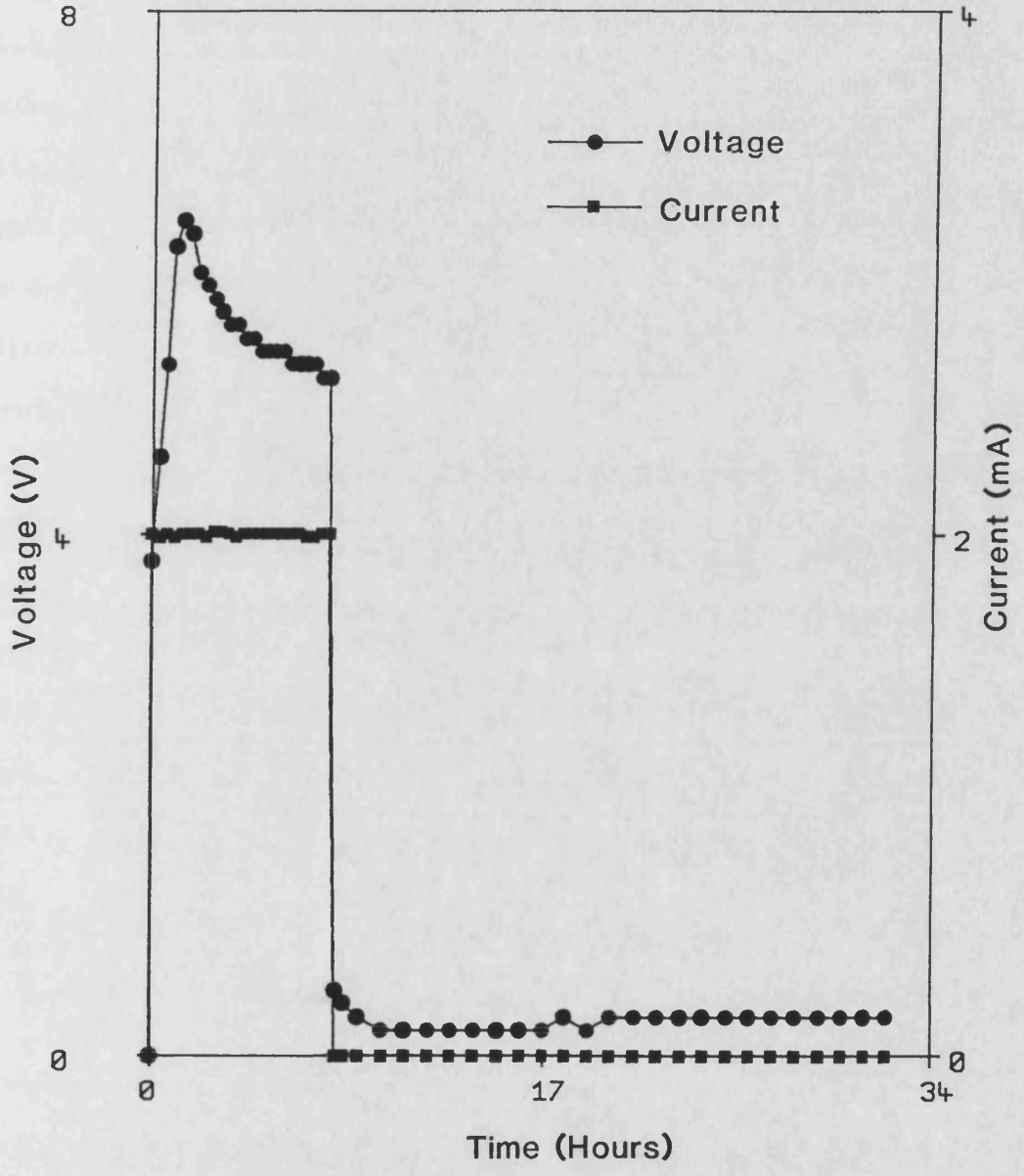


Figure 3.23. Changes in voltage measured during electrophoresis at a current of 2 mA applied during the diffusion lag time.

effected during steady state diffusion, peaking at 6.4 V, the expected voltage being approximately 4.5 V. These findings suggest a greater resistance to passage of an electrophoretic current prior to steady state diffusion having been attained. This effect is not fully understood, however it may be related to the PHEMA not having attained equilibrium conditions with propranolol HCl, thus on application of an electrophoretic current there is an additional retarding force imposed by the affinity of drug for the polymer. This effect may not be important at later times when the polymer is loaded with drug.

On removal of the electrophoretic current, the build up of propranolol HCl in the receptor appears to carry on as if the lag period had merely been interrupted by the electrophoretic current, the delivery rate decreasing slightly from that which would be expected during the lag time. From previous experiments on the diffusion of propranolol HCl through 1%C PHEMA, during the lag phase (section 3.3.3), between the times of 8 and 32 hours, the increase in mass of propranolol HCl in the receptor was found to be approximately 2.94 mg, in this present study it was found to be 2.07 mg.

The results in this study clearly indicate that a significant difference between drug delivery rates is produced by application of an electrophoretic current before and after steady state diffusion has been attained. Interestingly the voltage-time profile produced in this study follows a similar pattern to that found in the work of Lescure [128], where a constant current was applied from the start of experiments (without waiting for a lag time), a peak voltage corresponding to the lag time of bovine serum albumin appearance was found. The results in this study may also explain some of the

findings of Kumar [127], where the drug delivery rate appeared to be virtually zero during periods of no electrophoresis. This was probably due to the fact that experiments were all performed during the lag phase of diffusion (section 3.3.3), which may also explain why different measured rates of drug delivery were obtained when a constant voltage was applied at different stages of an experiment. It could be speculated that voltage-time profiles during the application of a constant current across polymer films may give information on whether a charged species has attained steady state diffusion or not.

3.3.11 Delivery of Propranolol HCl through Crosslinked PHEMA by a Programmable Feedback Electrophoresis System

The method used to estimate the drug delivery rate based on 10 samples at 3 minute intervals was checked during the periods of diffusion prior to electrophoresis where the delivery rate was constant. The mean of the computer-estimated drug delivery rate over a period of four hours was found to be within 3% of the delivery rate calculated from the concentration and time data stored by the computer. This was found to be the case for three successive experiments and thus the routine was assumed to be a satisfactory estimate of the drug delivery rate.

In all the initial feedback experiments based on the simple turning on and off of the power supply, during the period of feedback electrophoresis there was a slight undulation in the delivery rate, however over the eight hour period a zero-order type delivery rate was apparent. Figure 3.24 shows the type of relationship obtained between concentration and time, the example shown being for a programmed rate of 0.8 mg/h. Figure 3.25 shows the current changes

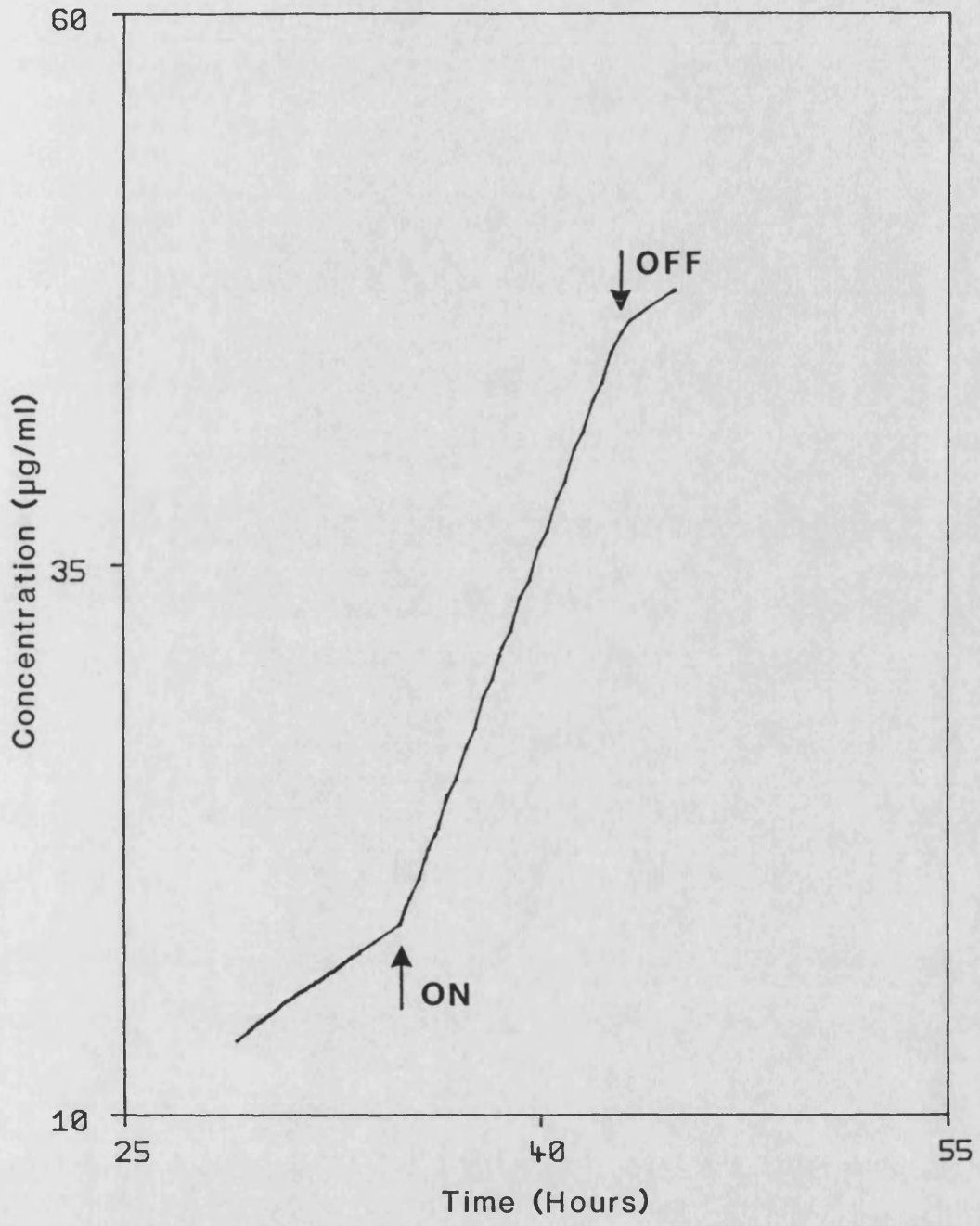


Figure 3.24. Changes in propranolol HCl concentration with time during feedback electrophoresis using a programmed delivery rate of 0.8 mg/h.

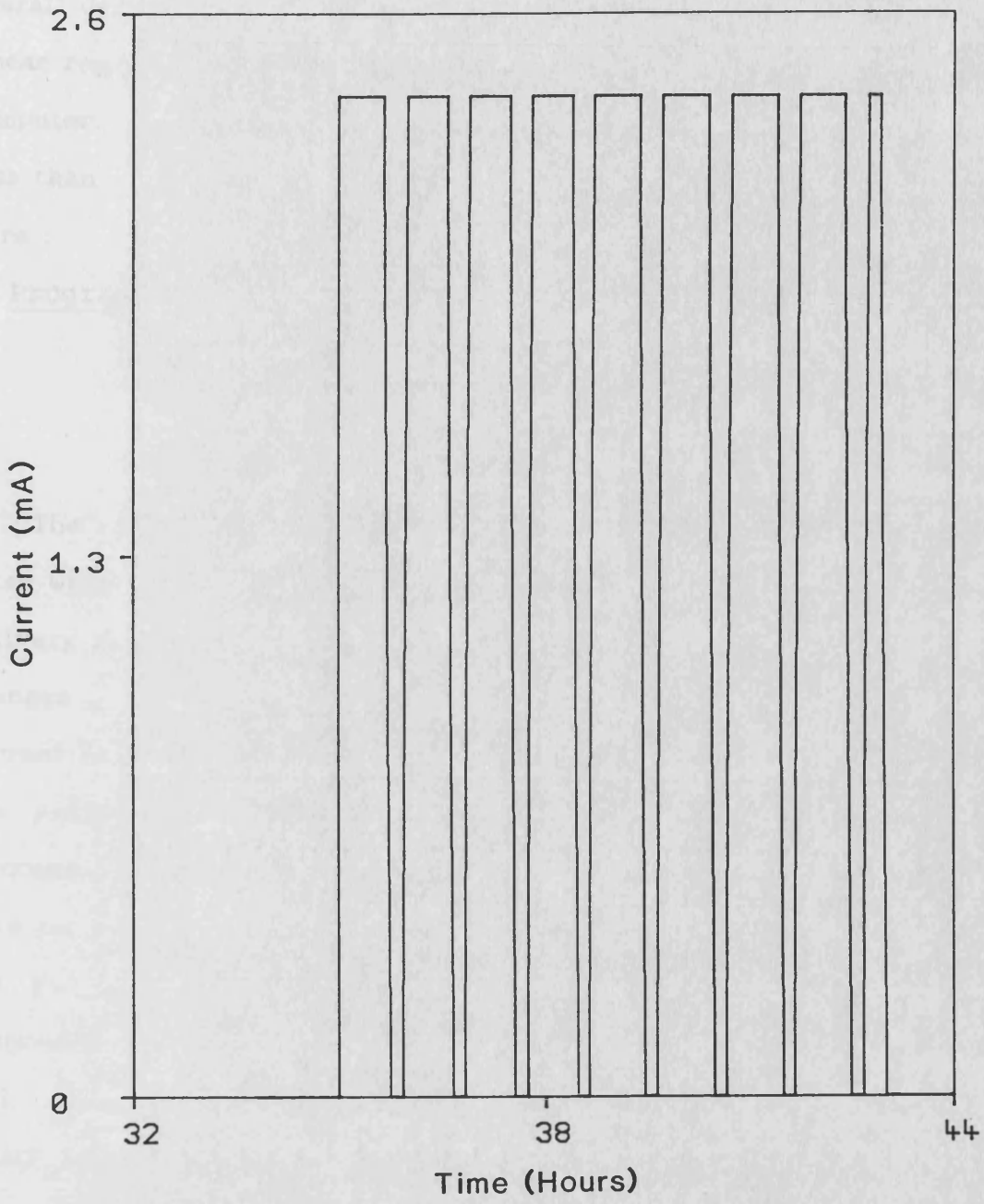


Figure 3.25. Variation of current during the feedback electrophoresis experiment shown in figure 3.24.

during the feedback period. For all the experiments performed the overall delivery rate during the feedback period was calculated using linear regression of the receptor concentration data collected by the computer. The results showed that the delivery rates obtained were less than the programmed desired delivery rates, the values obtained were :

Programmed Delivery Rate (mg/h)	Actual Delivery Rate (mg/h)
0.6	0.578
0.8	0.711
1.0	0.886

The discrepancies between the programmed and actual delivery rates were thought to be related to the method used to estimate the delivery rate. The method used was simple but insensitive to sudden changes in drug delivery rates, as occurs when an electrophoretic current is applied. This may explain why the discrepancy increased as the programmed delivery rate was increased. In order to reduce the discrepancies, a number of different sampling sizes and frequencies were tested, and the effect examined on the actual delivery rate when the programmed delivery rate was 0.8 mg/h. The computer was programmed to record all changes in the estimated drug delivery rate with respect to time. This enabled all changes to be examined in detail. The results obtained were as follows :

Sample Size and Frequency	Actual Delivery Rate (mg/h)
10 samples at 3 minute intervals	0.711
10 samples at 5 minute intervals	0.718
5 samples at 10 minute intervals	0.669
5 samples at 6 minute intervals	0.692

The results showed that there was not much difference between

the various sampling routines, however for future experiments the routine was changed to that based on 10 samples at 5 minute intervals.

The second feedback system examined was based on altering the electrophoretic current in a more controlled manner rather than on a simple on/off basis. The effect was to produce an actual delivery rate closer to the programmed value. Figure 3.26 shows the changes in concentration that occur during the experiment, once again an undulating pattern of delivery during feedback control was observed. The calculated delivery rate was 0.757 mg/h. The changes in current that occurred during feedback control are shown in figure 3.27. Figure 3.28 shows the relationship between receptor propranolol HCl concentration, electrophoretic current and computer estimated delivery rate to one another with respect to time. The insensitivity of the method used to estimate the drug delivery rate is demonstrated by the lag time required to detect actual changes in the delivery rate. The undulating changes in concentration with time during feedback are probably due to this lag time.

In this part of the study it has been shown that even using a very simple feedback model, an electrophoretic drug delivery device has great potential as a feedback controlled system. It can be envisaged that such an electrophoretically modulated delivery device with feedback control could act as a synthetic gland, containing, for example insulin where release was dependant on data received from a reliable glucose biosensor.

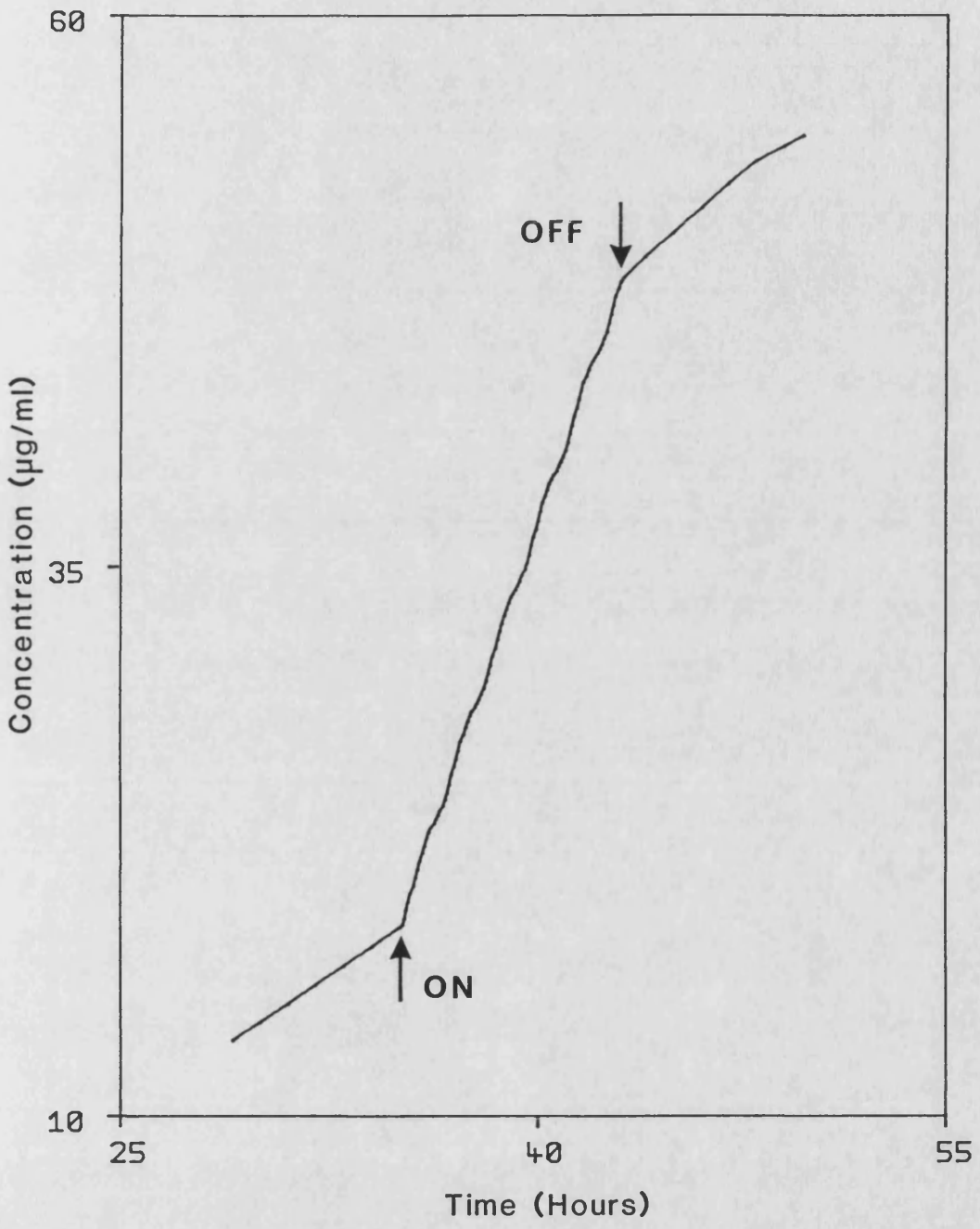


Figure 3.26. Changes in propranolol HCl concentration with time during feedback electrophoresis using a programmed delivery rate of 0.8 mg/h.

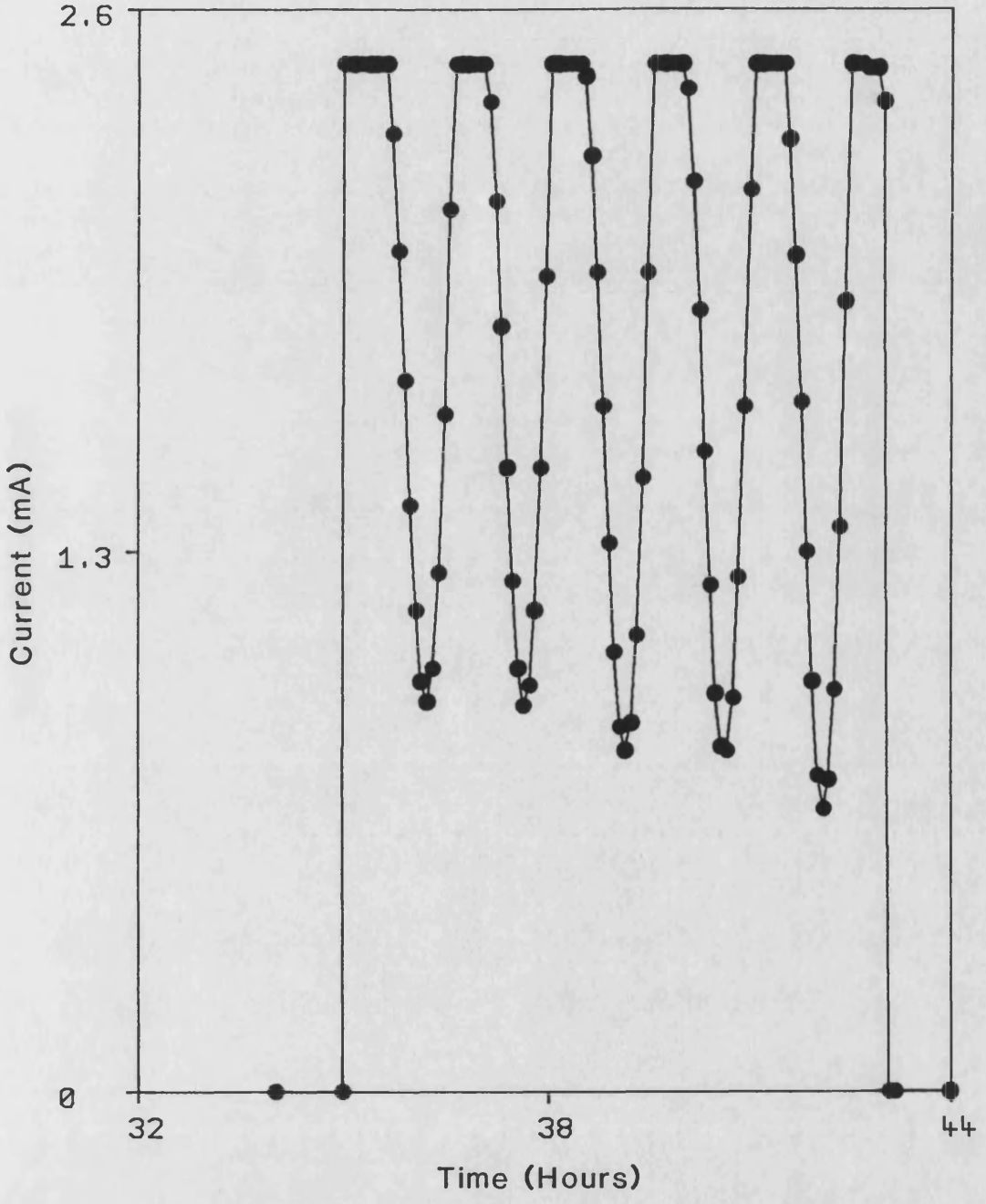


Figure 3.27. Variation of current during the feedback electrophoresis experiment shown in figure 3.26.

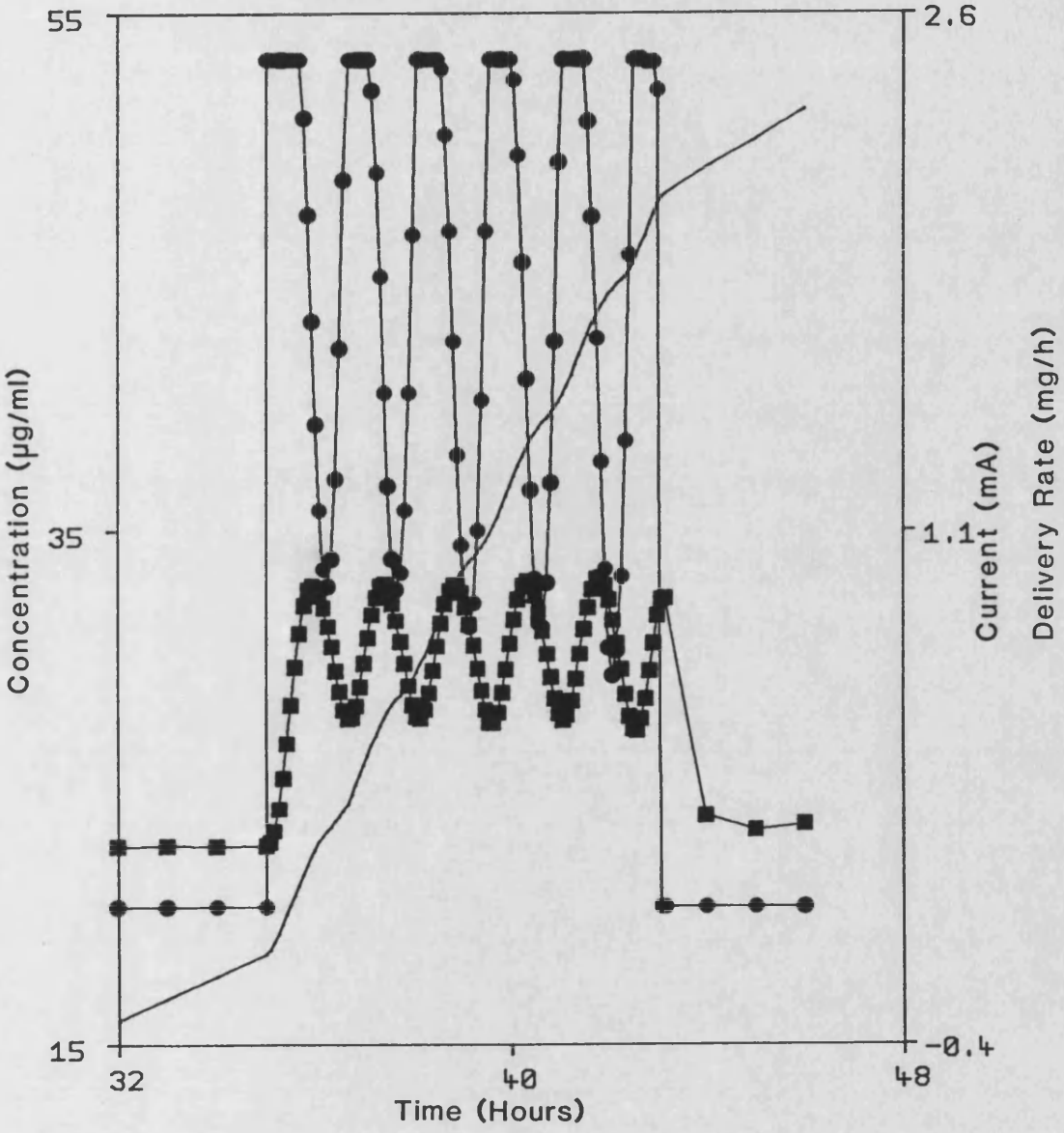


Figure 3.28. The relationship between concentration (——), current (—●—), and computer estimated delivery rate (—■—) with time for the feedback electrophoresis experiment shown in figure 3.26.

Chapter 4

An *In Vitro* Model of a Transdermal Electrophoretic Drug Delivery Device

4.1 Materials

Hairless mice, MF1/Hr.Hr, male, 13-23 weeks, from a nucleus stock obtained from Harlan Olac Ltd., Bicester, U.K., were supplied by the Animal House, University of Bath, Bath, U.K.

Tritiated water, stated specific activity 80 $\mu Ci\ ml^{-1}$, supplied by Amersham International plc, Little Chalfont, U.K. Batch No. LA 8451.

OptiPhase Safe (scintillation liquid), supplied by Fisons plc, Loughborough, U.K.

Sodium dodecyl sulphate, specially purified for biochemical work, supplied by BDH Ltd., Poole, U.K. Batch No. 512623OH.

Azone, pharmaceutical purity, was received as a gift from Nelson Research and Development, Irvine, CA, U.S.A. Batch No. 0923Q0312.

Polyoxyethylene 20 sorbitan monolaurate (Tween 20), supplied by Sigma Chemical Company Ltd., Poole, U.K. Batch No. 34C-1690.

For all other materials used see section 2.1 and section 3.1.

4.2 Methods

In this chapter the transport of propranolol HCl through hairless mouse skin will be examined, together with methods for the possible enhancement of this transport. The information derived from these experiments will be used in the investigation of the performance of an *in vitro* model of a transdermal electrophoretic drug delivery device using hairless mouse skin. The ultimate aim is to investigate whether controlled drug delivery may be achieved from this type of device.

4.2.1 Measurement of the Permeability Coefficient of Propranolol HCl through Hairless Mouse Skin

4.2.1.1 Design of Skin Diffusion Cell

The measurement of the permeability coefficients of water and propranolol HCl across hairless mouse skin was based on the infinite dose technique (section 1.5.3). A number of designs of skin diffusion cells have been used in this type of study, however many of the cells used are based on the Franz diffusion cell [26,189,201]. The cells manufactured for this study were made of glass and based on the Franz cell, a schematic diagram of the cell is shown in figure 4.1. The cell is composed of a donor compartment and a receptor compartment. The cell was prepared for experiments by firstly smearing a thin layer of vacuum grease (Apiezon N) onto the interfacial ground glass joints of the donor and receptor compartments. A magnetic stirring bar, 0.2 x 0.2 x 0.7 cm, was then placed into the receptor compartment. A circular stainless steel wire mesh was placed into a groove in the receptor compartment which provided a flat supporting surface for skin samples. Fresh hairless mouse skin samples were obtained for experiments. The mice were sacrificed by cervical dislocation, and an area of approximately 4 x 5 cm of whole-thickness abdominal skin removed. The sample of skin was gently stretched and placed onto the surface of the receptor compartment, with the dermal side facing into the receptor compartment. The donor compartment was placed on top of the receptor and the the excess skin was then trimmed using scissors before the whole cell was clamped together. The effective diffusional area of the skin was 1.77 cm^2. Into the receptor compartment was placed 4 ml of acetate buffer (appendix 2),

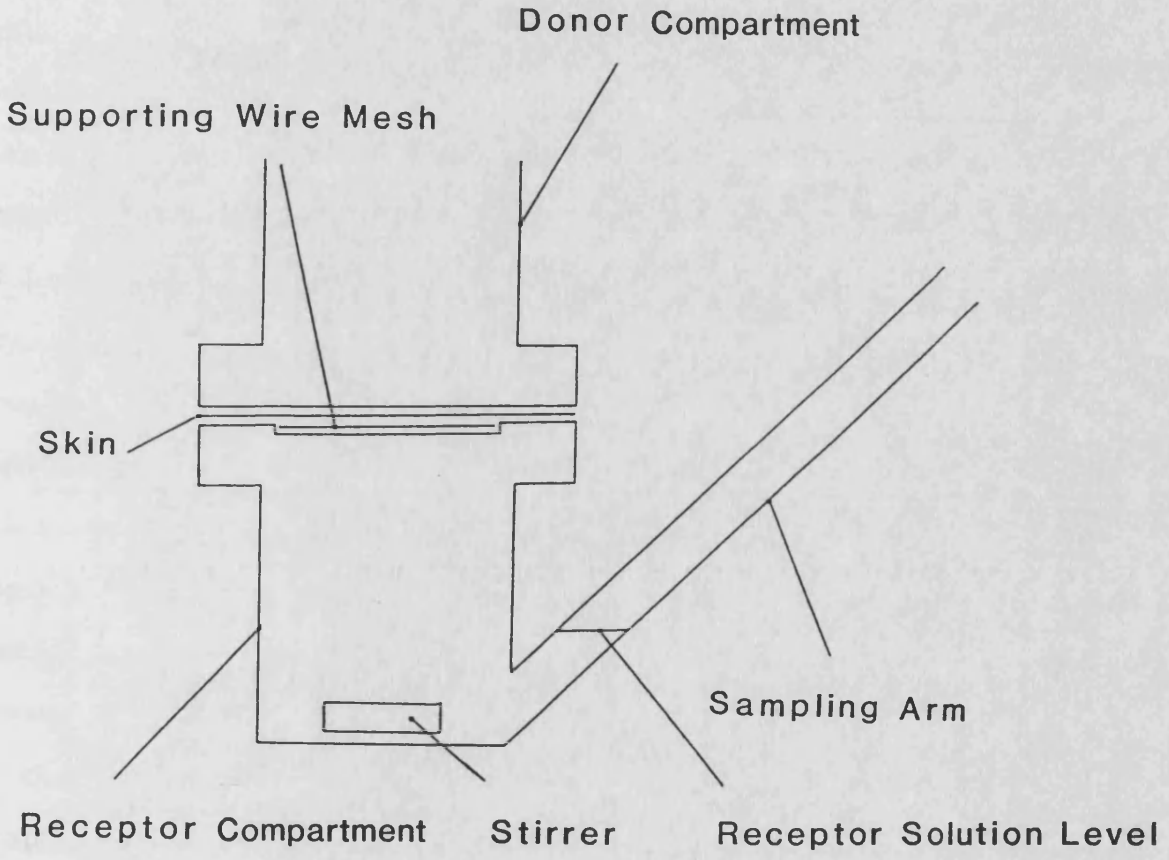


Figure 4.1. Schematic diagram of the diffusion cell used to study the permeation of propranolol HCl through hairless mouse skin.

this was slowly pipetted into the inverted diffusion cell, ensuring no air bubbles were trapped in the receptor compartment. The 4 ml of buffer ensured that the receptor was completely filled, with only a short length of the sampling arm containing buffer (this level was maintained by surface tension). The reason for not filling the sampling arm was due to poor mixing effects in this region (see below). The cell was then placed onto an underwater air-driven magnetic stirrer (Jencons Scientific Ltd., Leighton Buzzard, U.K.). The level of the magnetic stirrer was such that when the skin cell was placed on its surface, the water bath level was just below the interface of the donor and receptor compartments. The stirring rate of the magnetic stirrer was maintained constant by connecting a pressure gauge into the air-line and ensuring that a constant air pressure was maintained during experiments. The water bath was maintained at 30°C for all permeation experiments. The skin cell was allowed to equilibrate for 15 minutes, after which 3 ml of an appropriate donor solution was placed into the donor compartment and timing commenced. A square microscope slide, smeared with vacuum grease (Apiezon N), was placed onto the donor compartment to prevent a change in the concentration of the donor solution due to evaporation.

Initial experiments using tritiated water in the skin diffusion cell were as described above with the exception that 5 ml of buffer was present in the receptor compartment, which resulted in the sampling arm containing a significant volume of buffer. Though apparently satisfactory results were obtained with tritiated water using this volume (section 4.3.1.2), extremely low and erratic permeability data were produced with propranolol HCl. It was thought

that this may have been the result of poor mixing in the sampling arm, which was further investigated in subsequent experiments. The skin diffusion cell was prepared as described above using a plastic sealing film (Nesco Film) to replace the skin sample. A crystal of potassium permanganate was placed into the receptor compartment together with the magnetic stirring bar. The experiment was repeated with both 4 ml and 5 ml of buffer present in the receptor compartment, and the change in colouration of the receptor compartment observed.

4.2.1.2 Permeation of Tritiated Water through Hairless Mouse Skin

In order to ensure that the method used to determine the permeability of propranolol HCl through hairless mouse skin maintained the skins integrity (section 4.2.1.3), the permeability of tritiated water through hairless mouse skin was determined and compared with literature values. The permeation of tritiated water through hairless mouse skin was examined according to the method described in section 4.2.1.1. The skin cell was assembled as described, with 5 ml of acetate buffer in the receptor compartment, and after allowing the cell to equilibrate, 3 ml of tritiated water with a stated specific activity of 80 $\mu Ci\ ml^{-1}$ was placed into the donor compartment and timing started. Samples of 0.2 ml were removed from the receptor compartment at approximately 50 minute intervals up to a time of approximately five hours and retained for assay using a liquid scintillation counting technique. The samples were removed using a calibrated automated pipette (P200, Gilson) and after each sample was removed, 0.2 ml of acetate buffer was pipetted back into the receptor compartment. The removed samples were placed into 5 ml

polyethylene scintillation counting vials (LKB Wallac Oy, Turku, Finland) and 4 ml of scintillation liquid measured using an automatic pipette (Oxford Pipettor 400/401) into each of the vials. The scintillation cocktail used (Optiphase Safe) was suitable for the incorporation of aqueous samples. The vials were capped and vortex mixed (Whirlimixer, Fisons Scientific Apparatus, Loughborough, U.K.) for approximately 10 seconds before being placed into glass scintillation vials (LKB). The samples were counted in a liquid scintillation counter (Rackbeta 1215, LKB Wallac Oy), employing a predetermined quench curve (see below). Each sample was counted in duplicate for one minute and an average value taken. A background count was subtracted from all counts. The external standard ratio time was 30 seconds. After correcting for dilution, the permeability coefficient of tritiated water through hairless mouse skin was determined from the gradient of the linear part of a plot of the cumulative amount of tritiated water in the receptor compartment versus time. Six replicates of the experiment were performed. The specific activity of the stock tritiated water (stated activity 80 $\mu Ci\ ml^{-1}$) was also determined.

A quench curve was constructed using the external standards ratio method. Into each of ten empty 5 ml polyethylene scintillation counting vials was placed 0.2 ml of tritiated water with an activity of 0.88 $\mu Ci\ ml^{-1}$ and 4 ml of scintillation liquid (Oxford Pipettor 400/401). Vial 1 was capped. To vials 2 to 10 was added 5, 10, 15, 20, 30, 50, 75, 100, and 150 µl of carbon tetrachloride respectively; the carbon tetrachloride acted as a quenching agent. The vials were vortex mixed and then loaded into the liquid scintillation counter which was programmed to count and construct a quench curve by means

of an external standard source, the quench curve is shown in figure 4.2. The quench curve, which is expressed as counting efficiency versus external standard ratio, was stored in the instruments memory. For all subsequent samples the scintillation counter determined the counts per minute and the degree of quenching for an unknown sample. Using the external standard ratio and quench curve, the efficiency is calculated and the liquid scintillation counter thus produces a value for the disintegrations per minute (dpm).

In order to demonstrate that a linear relationship existed between the measured dpm and tritiated water specific activity, a series of dilutions from a stock solution (0.88 μCi ml^{-1}) were prepared and the resulting solutions assayed for tritium activity.

4.2.1.3 Permeation of Propranolol HCl through Hairless Mouse Skin

The permeation of propranolol HCl through hairless mouse skin was examined according to the method described in section 4.2.1.1. The skin cell was assembled as described, and after allowing the cell to equilibrate, 3 ml of an approximately 8.4 mM solution of propranolol HCl in acetate buffer (appendix 2) was placed into the donor compartment and timing started. Samples of 0.2 ml were removed from the receptor compartment at approximately 16, 18, 20, 22 and 24 hours. The samples were removed using a calibrated automated pipette (P200, Gilson) and after each sample was removed, 0.2 ml of acetate buffer was pipetted back into the receptor compartment. The removed samples were placed into 0.3 ml HPLC vials (Chromacol Ltd., London, U.K.) and capped using a crimper. The samples were stored at 4°C until ready for assay. Six replicates of the experiment were performed. Assay of the samples was by HPLC (section 2.2.2). After

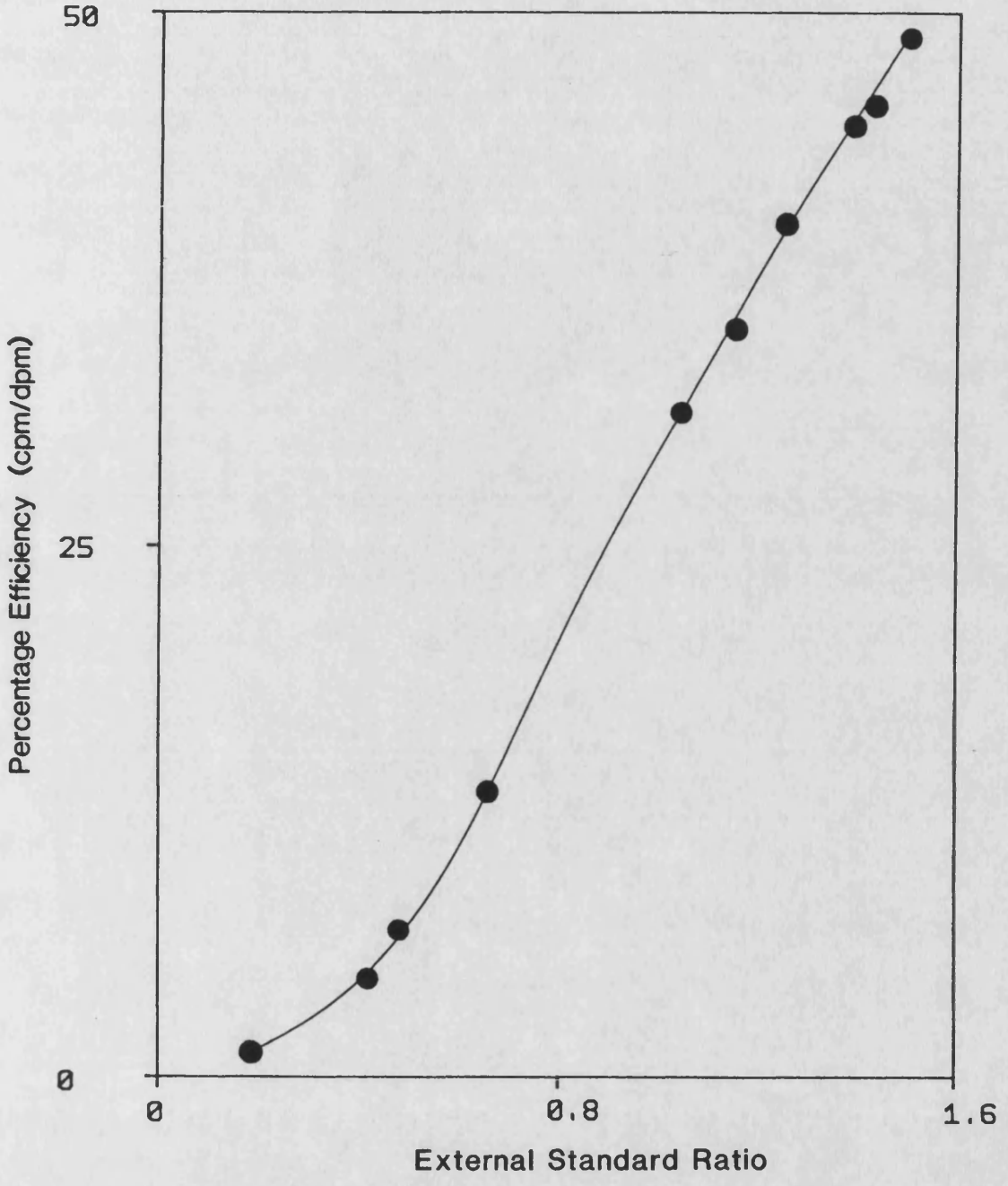


Figure 4.2. Quench curve for tritiated water in Optiphase Safe.

correcting for dilution, the concentration of propranolol HCl in the receptor compartment at each sampling time was calculated, and from the gradient of the linear part of a plot of the cumulative molar concentration of propranolol HCl in the receptor compartment versus time the permeability coefficient of propranolol HCl through hairless mouse skin was calculated (section 1.5.3).

4.2.2 Effect of Penetration Enhancers on the Permeation of Propranolol HCl through Hairless Mouse Skin

The transport of ionised drugs across the stratum corneum by passive diffusion is very slow due to unfavourable partitioning into this lipophilic membrane [169]. Ionised drug transport may be enhanced by a number of methods as described above in sections 1.3.8.1, 1.3.9.1, 1.5.1, and 1.5.2. In the present study just two of these methods were investigated. The first method studied was to increase the lipophilicity of the ionised permeant by ion-pair formation [168,169,278,279]. In this study the use of sodium dodecyl sulphate as an ion pair for propranolol HCl was investigated. The second method studied here by which the transport of charged species may be enhanced was to disrupt the skin structure by means of a penetration enhancer (section 1.5.2), thereby reducing the stratum corneum resistance. The use of Azone as such an agent was investigated.

4.2.2.1 Sodium Dodecyl Sulphate (SDDS) as an Ion-Pairing Agent

The interaction between large organic anions and cations in aqueous solution leading to the formation of ion-pairs has been demonstrated by a number of workers [280,281]. It is believed that

the forces holding ion-pairs together are a combination of electrostatic and hydrophobic interactions [281]. One of the intrinsic properties of ion-pair systems is low aqueous solubility [278]. This was found in the present study when an equimolar solution of approximately 8.4 mM of SDDS and propranolol HCl was prepared in acetate buffer (appendix 2). A white, cloudy precipitate was formed, which also occurred when the same solution was prepared in double distilled water rather than buffer. The interaction between SDDS and a number of organic cations has previously been described [280,281], these systems showing complex interactions in aqueous solution. If a low concentration of an ion-pairing organic cation is taken and small volumes of a concentrated solution of SDDS added then a number of changes may be observed as the concentration of SDDS increases. Initially a clear region exists where soluble ion-pairs between SDDS and the organic cation exist in equilibrium with the homogeneous solution of the various other dissociated and non-dissociated ions present. As the concentration of SDDS is increased a heterogeneous phase separation occurs, apparent by the appearance of turbidity, which is the point at which the solubility product of the ion-pair species is exceeded and a coacervate phase is formed. As the concentration of SDDS is increased further a stage will be attained where the system becomes clear again following the micellar solubilisation of the complex coacervate.

The concentration of ions present will therefore greatly affect the type of interaction occurring in ion-pairing systems, which will in turn be expected to affect the disposition of the ions involved. The effect of these ion-pair interactions on the transport of a model organic ion, cromoglycate, through a polyamide membrane has been

reported [279]. It was found that before the solubility product of the system had been exceeded, an increase in ion-pair formation resulted in an increase in cromoglycate flux, however once the formation of complex coacervate occurred, a marked reduction in cromoglycate flux resulted, thought to be due to a decrease in the thermodynamic activities of cromoglycate and its ion pair. In the present study the effect of SDDS on the transport of propranolol HCl across hairless mouse skin was examined. Two systems were investigated, firstly the case where the concentration of the ions present were such that the solubility product was not exceeded. The second system examined, as a control, was where the complex coacervate was solubilised by a sufficient concentration of SDDS.

4.2.2.1.1 Determination of the Apparent Solubility Product of the Complex between SDDS and Propranolol HCl

The determination of the apparent solubility product (K'_{sp}) of the complex between SDDS and propranolol HCl in both distilled water and acetate buffer was based on a combination of a static conductimetric and turbidimetric titration [280,281]. If a concentrated solution of surfactant is added in increasing amounts to a dilute solution of an organic ion-pair, a progressive increase in conductivity occurs as the concentration of surfactant increases until the K'_{sp} is attained, this end-point is associated with a marked change in the slope of the conductivity curve, together with the appearance of turbidity. The K'_{sp} of the complex between SDDS and propranolol HCl in double distilled water was determined by both conductimetric and turbidimetric methods. The K'_{sp} of the complex would be expected to change in the presence of acetate buffer

[280,282], however conductimetric methods can not be used at higher ionic strengths since the change in conductivity measured with increasing concentrations of surfactant are small relative to the high background conductivity. Thus a turbidimetric method was used to estimate the K'_{sp} in buffer.

The SDDS used in this study was obtained as a specially purified sample, however it is known that hydrolysis may occur upon storage [280,281], therefore the sample was further purified immediately before use. Soxhlet extraction was performed with petroleum ether for 48 hours at approximately 50°C. The sample was dried at room temperature in a vacuum oven and recrystallized twice from absolute ethanol. The resulting crystals were then dried in a vacuum oven at 40°C to constant weight at a pressure of approximately 900 mBar.

Double distilled water from an all-glass still was used in this study. The double distilled water was freshly prepared approximately 24 hours before use, and was stored in a specially reserved glass container. All glassware used in this study was aged by soaking in a concentrated solution of SDDS overnight and thoroughly rinsing with distilled water several times and then finally with double distilled water before drying in an oven.

The apparatus used for measuring conductivity consisted of a jacketed beaker (figure 4.3), whose temperature was maintained constant by water circulated from a water bath. The conductivity of the system was measured using a glass-platinum Mullard dip cell, in conjunction with a conductivity bridge (Wayne Kerr B642, Wayne Kerr Company Ltd., New Malden, U.K.). The magnetic stirrer was only used to agitate the sample solution until the temperature had equilibrated, stirring was stopped whilst readings were taken as the

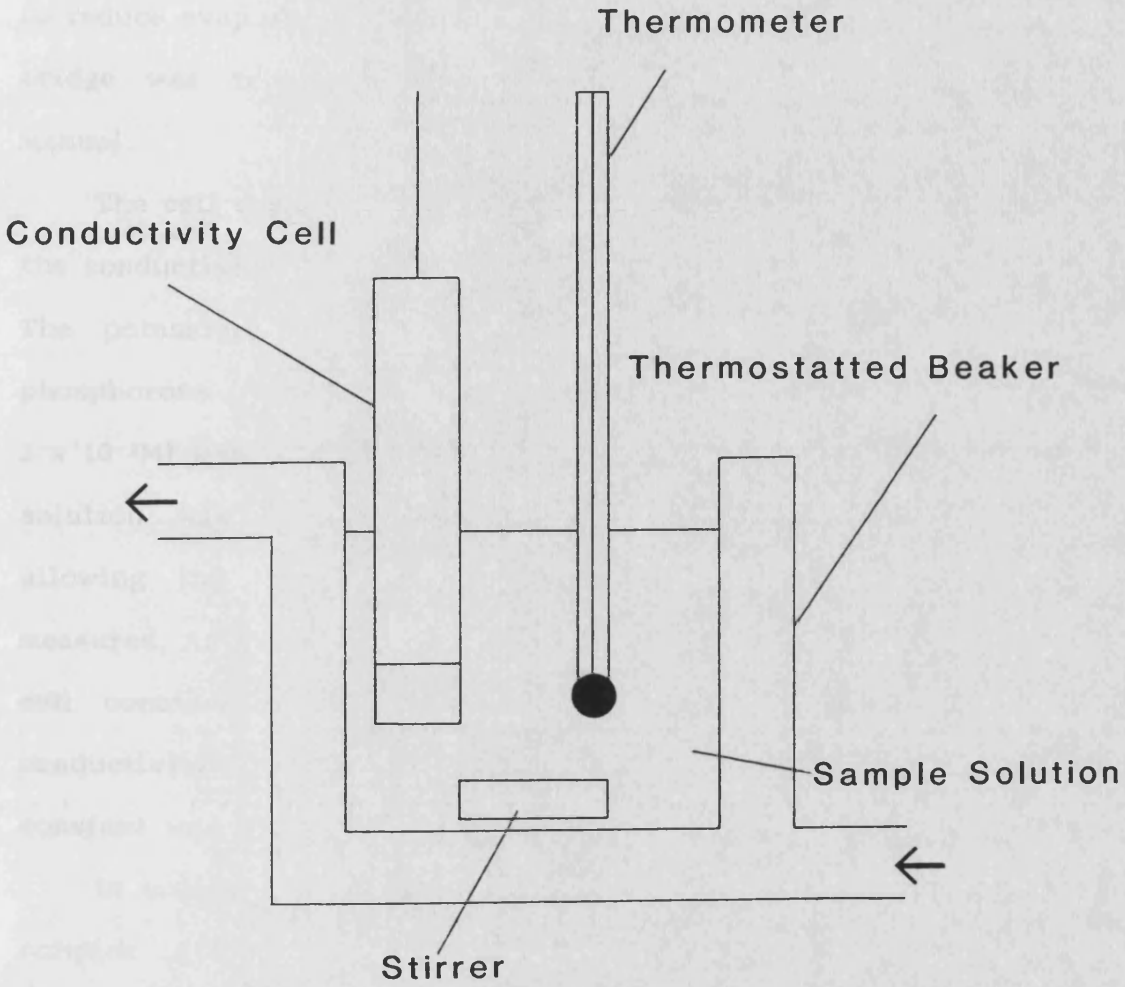


Figure 4.3. Schematic diagram of the jacketed beaker in which conductivity was measured.

stirrer was found to interfere with the conductivity bridge. Whilst allowing the solutions to attain equilibrium temperature, the jacketed beaker was carefully covered with a layer of aluminium foil to reduce evaporation. Prior to any measurements the conductivity bridge was trimmed and calibrated as described in the instrument manual.

The cell constant of the dip cell was determined by measuring the conductivity of a range of potassium chloride solutions at 25°C. The potassium chloride used was dried at 60°C in an oven over phosphorous pentoxide for 24 hours. A range of solutions (5×10^{-4} to 2×10^{-2}M) were prepared in double distilled water. 50 ml of each solution was in turn placed in the jacketed beaker, and after allowing the temperature to equilibrate to 25°C the conductivity measured. After correcting for the conductivity of water, the derived cell constant was determined from literature values of specific conductivity [283], the results shown in table 4.1. The mean cell constant was found to be 1.41 cm^{-1}.

In order to determine the stoichiometry and the K'_{sp} of the complex between SDDS and propranolol HCl, the K'_{sp} was determined for three different concentrations of propranolol HCl in double distilled water, for each concentration a series of solutions were prepared with successive increments in the amount of SDDS. The three systems examined were :

1) 7.96×10^{-4}M propranolol HCl in double distilled water with the concentration of SDDS ranging from 0.3 to 1.6×10^{-4}M.

2) 5.04×10^{-4}M propranolol HCl in double distilled water with the concentration of SDDS ranging from 0.6 to 3.2×10^{-4}M.

3) 2.02×10^{-4}M propranolol HCl in double distilled water with the

Table 4.1 Calculated cell constants from conductivity readings and literature specific conductivity values for a range of potassium chloride solutions at 25°C.

Concentration ($M \times 10^3$)	Conductivity ($S \times 10^6$)	Corrected Conductivity ($S \times 10^6$)	Literature Specific Conductivity ($S\ cm^{-1} \times 10^6$)	Cell Constant (cm^{-1})
0	0.8	-	-	-
0.5	54.0	53.2	73.9	1.389
1.0	106.8	106.0	147.0	1.387
5.0	512.2	511.4	716.8	1.402
10.0	997.4	996.6	1412.7	1.418
20.0	1936.0	1935.2	2766.8	1.430

concentration of SDDS ranging from 2 to 8 x 10^{-4}M.
The solutions were prepared and equilibrated for 24 hours at $30°_{C}$. For each system the conductivity of 50 ml of each solution was measured in the jacketed beaker at a temperature of 30°C. The turbidity of each system was determined by measuring the apparent absorbance of the solutions in a 1 cm cuvette at 500 nm (UV-120-02, Shimadzu Corporation, Kyoto, Japan). The solutions were transferred to the cuvette and measured immediately to minimise temperature changes.

Solutions containing SDDS-propranolol HCl ion-pairs were examined by nuclear magnetic resonance (NMR) together with pure sample solutions of SDDS and propranolol HCl. A solution of 100 ml 0.1 M propranolol HCl and 0.01 M SDDS in distilled water gave a white precipitate of the ion-pair which was isolated by centrifugation, and the supernatant layer discarded. The ion-pair was dissolved in 150 ml of chloroform and washed with distilled water (3 x 10 ml). The organic layer was evaporated in vacuo to leave a white waxy solid. This was dried in a vacuum oven at room temperature at a pressure of approximately 900 mBar and the solid dissolved in $CDCl_3$ subjected to NMR analysis (appendix 5).

The K'_{sp} of the ion-pair complex in acetate buffer at 30°C was estimated using the turbidimetric method as described above on a series of solutions containing 7.98 x 10^{-4}M propranolol HCl in acetate buffer, with the concentration of SDDS varying from 0.5 to 3.2 x 10^{-4}M.

4.2.2.1.2 Determination of the Concentration of SDDS Required to Solubilise the Complex between SDDS and Propranolol HCl

The determination of the concentration of SDDS required to solubilise the complex between SDDS and propranolol HCl was by a turbidimetric method [281]. A series of solutions of 8.45×10^{-3}M propranolol HCl in acetate buffer were prepared with the concentration of SDDS varying from 0.5 to 3.2×10^{-4}M. The solutions were equilibrated at a temperature of 30°C for 24 hours, and the apparent absorbance of the solutions measured as described in section 4.2.2.1.1.

4.2.2.1.3 Effect of SDDS on the Permeation of Propranolol HCl through Hairless Mouse Skin

The effect of SDDS on the permeability of hairless mouse skin to propranolol HCl was investigated. The method used was similar to that in section 4.2.1.3 except for the composition of the donor compartment solution. The donor compartment contained 3 ml of approximately 8.4 mM propranolol HCl in acetate buffer in the presence of either 9.40×10^{-6}M SDDS or 2.69×10^{-2}M SDDS. The former concentration of SDDS was chosen as it is just below that which would be expected to exceed the K'_{sp} of the complex between SDDS and propranolol HCl at 30°C (section 4.3.2.1.1). Six replicates of the permeation experiment were performed at this concentration of SDDS. The higher concentration of SDDS represents the estimated value necessary to solubilise the complex between SDDS and propranolol HCl at 30°C (section 4.3.2.1.2). Three replicates of the permeation experiment were performed at this concentration of SDDS.

4.2.2.2 Effect of Azone on the Permeation of Propranolol HCl through Hairless Mouse Skin

The effect of the penetration enhancer Azone on the permeability coefficient of propranolol HCl was investigated. The method used was similar to that described above in section 4.2.1.3 except for the composition of the donor compartment solution. The donor compartment contained 3 ml of approximately 8.4 mM propranolol HCl in one of the following formulations :

A - 2% w/v Azone in acetate buffer (appendix 2)

B - 0.11% w/v Tween 20 in acetate buffer

C - 1% w/v Azone and 0.11% w/v Tween 20 in acetate buffer

D - 2% w/v Azone and 0.11% w/v Tween 20 in acetate buffer

E - 3% w/v Azone and 0.11% w/v Tween 20 in acetate buffer

F - 5% w/v Azone and 0.11% w/v Tween 20 in acetate buffer

G - 10% w/v Azone and 0.11% w/v Tween 20 in acetate buffer

Formulation A was used as a control since it has been reported that Azone alone when mixed in solution may have some enhancement effect [176]. The rest of the formulations used Tween 20 to form an emulsion of Azone in acetate buffer [284], formulation B acting as a control. Six replicate skin permeation experiments were performed for each formulation. The sampling, assaying and calculation of permeability coefficients were as described in section 4.2.1.3.

4.2.3 In Vitro Model of a Transdermal Electrophoretic Device

In this section an in vitro model of a transdermal electrophoretic delivery device using hairless mouse skin is investigated. It has been demonstrated by work reported in chapter 3 that constant current electrophoresis can be used to control the

transport of propranolol HCl through crosslinked PHEMA films. It will also be seen from results reported subsequently in section 4.3.2.2 that the transport of propranolol HCl across hairless mouse skin in vitro is significantly enhanced using Azone. The information derived from these experiments was used to investigate the potential of a transdermal electrophoretic system as a controlled drug delivery device.

A schematic diagram of the model system used in this study is shown in figure 4.4, together with a photograph of the actual three compartment cell (figure 4.5). The design of the three compartment cell was based on the electrophoresis cell described in section 3.2.1. The receptor and drug reservoir compartments each had a capacity of approximately 220 ml, the cathode compartment having a capacity of approximately 3.5 ml. The separation of the uncoated platinum electrodes (section 3.2.1) in this system was 4.5 cm.

The assembly of the three compartment cell and preparatory experimental details are very similar to those described for the electrophoresis cell in section 3.2.1. 1%C PHEMA films having a thickness of approximately 0.1 cm were prepared as described in section 2.2.4.2, and discs of 4 cm diameter were cut. The discs were stored in pH 4.48 buffer at 4°C (appendix 2) prior to use. The interfacial ground glass joints of the three compartment cell were smeared with a thin layer of vacuum grease (Apiezon N). A section of hairless mouse skin was obtained (section 4.2.1.1) and placed over the interface of the receptor compartment, with the dermal side facing into the receptor. The exposed area of skin was 2.54 cm^2. The cathode compartment, with a platinum electrode in position, was carefully positioned onto the interface of the receptor compartment.

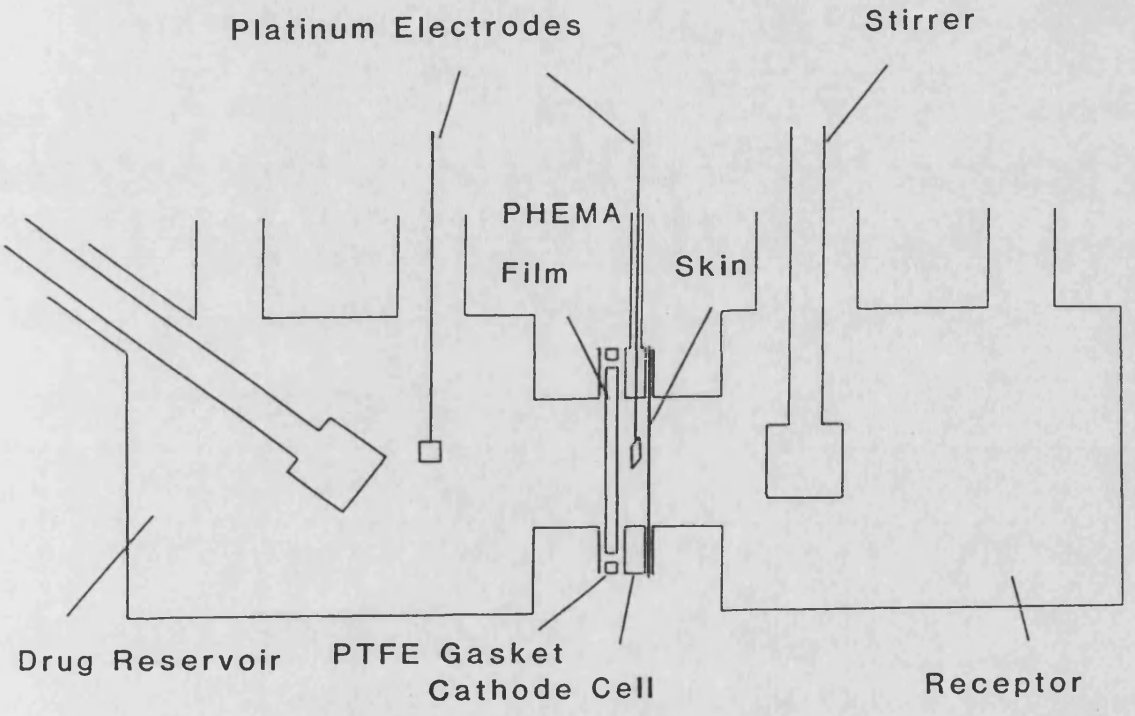


Figure 4.4. Schematic diagram of the three compartment model used to investigate a transdermal electrophoretic device.

Figure 4.5. Photograph of the three compartment model of a transdermal electrophoretic device.

An annular sheet of polytetrafluoroethylene (PTFE), with a thickness of 0.08 cm and internal diameter of 4 cm was placed at the exposed interface of the cathode compartment, and a disc of polymer film placed in its centre. The reservoir compartment was then assembled into position next to the cathode compartment and the three compartments clamped tightly. Into the cathode compartment was then placed one of two solutions :

1) 3.5 ml of acetate buffer (appendix 2)

2) 3.5 ml of 1% w/v Azone and 0.11% w/v Tween 20 in acetate buffer

Into the receptor compartment was placed 210 ml of pH 4.48 buffer, and into the drug reservoir was placed 200 ml of pH 4.48 buffer. The anode electrode was then inserted into the drug reservoir compartment. The whole cell was mounted in a specially constructed perspex stand and immersed in a water bath maintained at 25°C. The drug reservoir and receptor compartments were stirred continuously using perspex stirrers driven by 12 volt motors at 330 rev min^{-1} (RS Components Ltd., Corby, U.K.), which were powered by a constant voltage power supply (E30/1 Farnell Instruments Ltd, Wetherby, U.K.).

The cell was allowed to equilibrate for 30 minutes, after which 20 ml of a stock propranolol HCl solution in acetate buffer was added to the reservoir compartment to give a final concentration of approximately 14 mM (0.43%w/v), timing was then started. After 15 seconds a 10 ml sample was withdrawn from the reservoir compartment to give a measurement of the initial reservoir propranolol HCl concentration. This sample was analysed by u.v. spectrophotometry (section 2.2.1). The concentration of propranolol HCl in the receptor compartment was measured using a flow through u.v. detector system as described in section 3.2.1.

At the end of each experiment the hydrogel thickness was measured (section 2.3.4.2). The electrodes were thoroughly rinsed in distilled water, followed by double distilled water. The electrodes were then stored in fresh double distilled water until required.

Three experiments were performed, in each experiment the power supply was turned on to a constant current of 2 mA for two periods of 4 hours, the first period commencing at 57 hours and the second at 75 hours. The first experiment examined the appearance of propranolol HCl in the receptor compartment with just buffer in the cathode compartment. A second experiment examined the effect of an Azone emulsion (as described above) in the cathode compartment. A third experiment was performed as a control where no propranolol HCl was added to the drug reservoir, with an Azone emulsion present in the cathode compartment.

4.3 Results and Discussion

4.3.1 Measurement of the Permeability Coefficient of Propranolol HCl through Hairless Mouse Skin

4.3.1.1 Design of Skin Diffusion Cell

By investigating the mixing of a solution containing a crystal of potassium permanganate in the receptor compartment it was found that with 5 ml of buffer present the majority of the receptor attained a uniform purple colour within about 20 seconds, however the sampling arm was not evenly coloured. The purple colouration in the buffer became less intense with distance up the sampling arm indicating that mixing was not occurring in this area. In the study using 4 ml of buffer, uniform colouration occurred in all parts of the buffer within about 20 seconds. It was therefore assumed that uniform mixing was occurring in all parts of the cell, and 4 ml of receptor buffer was used in all skin transport studies using propranolol HCl. This mixing effect may explain why lower than expected values were obtained for the permeability coefficient of tritiated water where 5 ml of receptor solution was used in the studies (section 4.3.1.2).

4.3.1.2 Permeation of Tritiated Water through Hairless Mouse Skin

The specific activity of the stock tritiated water was determined as 73.6 $\mu Ci\ ml^{-1}$. The reproducibility of liquid scintillation counting was determined by preparing four dilutions of the stock solution of tritiated water to give values ranging from 3000 to 366000 disintegrations per minute. The coefficient of variation did not exceed $\pm$ 2.0% (n=5) for any of the samples, thus the reproducibility of the technique was considered satisfactory.

Figure 4.6 indicates that a linear relationship exists between relative count and tritiated water specific activity. Linear regression analysis of the line yielded the following statistics :

Number of points : 7

r^2 : 0.9989

Slope (S.D) : 1.1284 (0.0165)

Intercept (S.D) : -1.1803×10^{-2} (8.69×10^{-3})

It was therefore considered that liquid scintillation counting was a satisfactory assay method for the determination of tritiated water activity.

The permeability coefficient of tritiated water through hairless mouse skin at 30°C was calculated from the linear part of a plot of cumulative tritium activity in the receptor compartment versus time (section 1.5.3). An example of a plot is shown in figure 4.7. The mean value of the permeability coefficient of tritiated water through hairless mouse skin was calculated as 9.65×10^{-4} cm h^{-1} with a standard error of 0.75×10^{-4}. A reported value for the permeability coefficient of tritiated water through hairless mouse skin is 3.36×10^{-3} cm h^{-1} at 31°C [197], which is higher than the value obtained in the present study. The lower value obtained in the present study may be partly due to the different temperature used in the studies and partly due to mixing effects in the receptor compartment described above (section 4.3.1.1). Corresponding values for the permeability coefficient of tritiated water in human skin have been reported as 1.4×10^{-3} cm h^{-1} at 31°C [197], and also as varying between 1.2 and 1.5×10^{-3} cm h^{-1} depending on the skin sample [285], though no indication was given in the latter report as to the temperature at which these values were measured. The results however indicate that

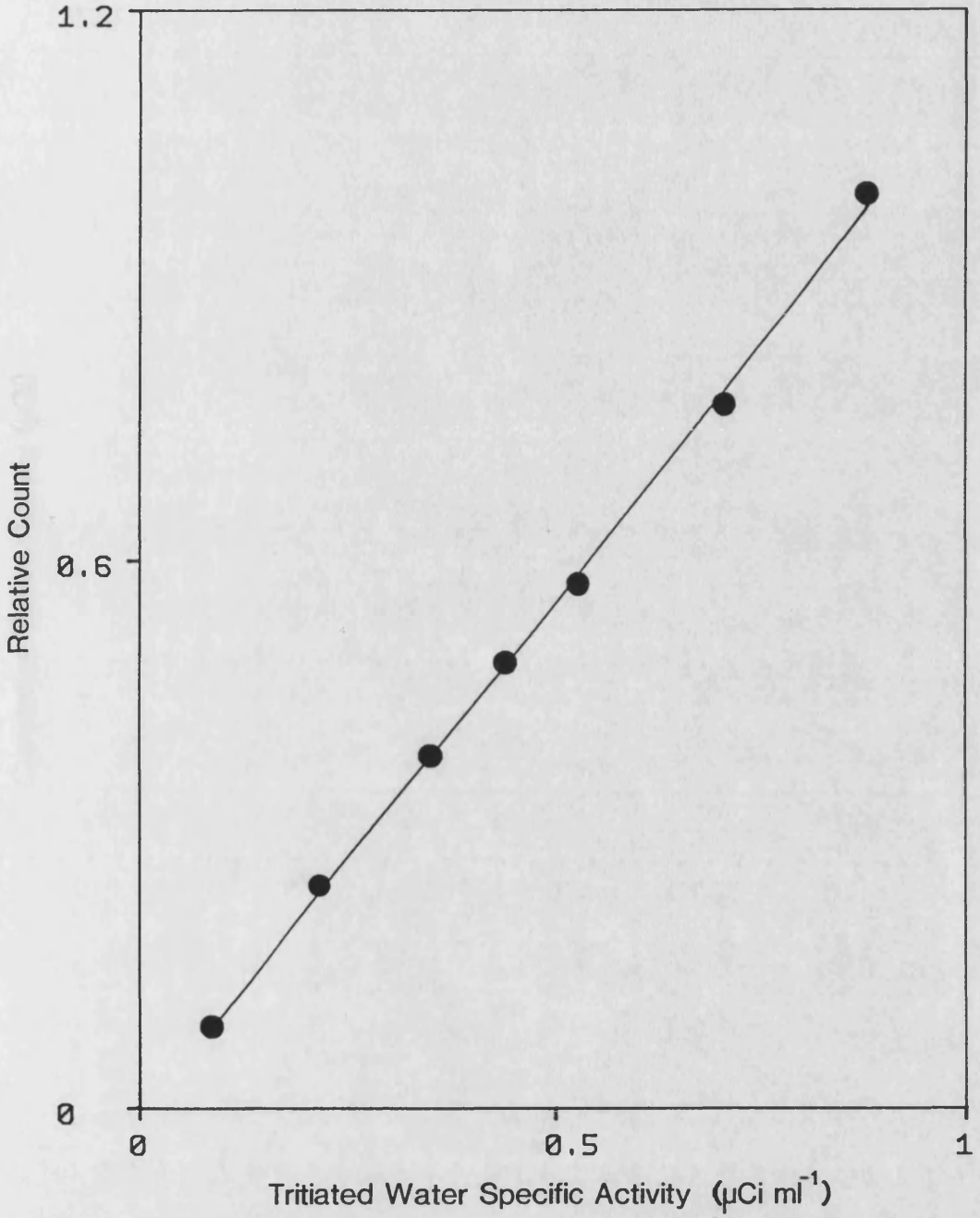


Figure 4.6. Calibration plot for the liquid scintillation counting assay of tritiated water.

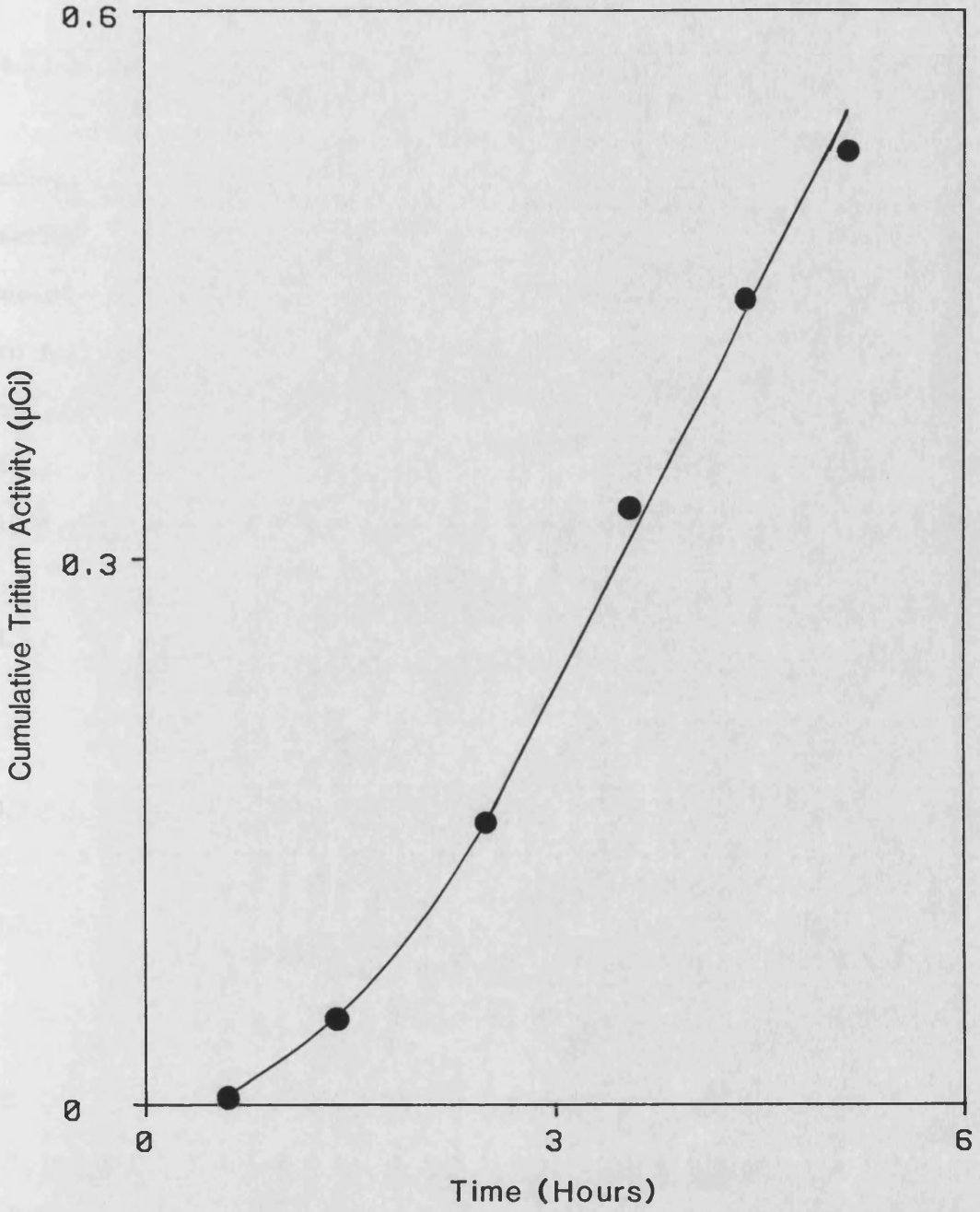


Figure 4.7. Relationship between cumulative tritium activity in the receptor compartment versus time.

the methodolgy used maintains the integrity of hairless mouse skin.

4.3.1.3 Permeation of Propranolol HCl through Hairless Mouse Skin

The cumulative amount of propranolol HCl in the receptor compartment (in moles) at each sampling time was calculated, and a series of plots of the cumulative amount of propranolol HCl (in moles) in the receptor compartment versus time were obtained similar to figure 4.7. The permeability coefficient of propranolol HCl was obtained from the linear portion of the plots as described in section 1.5.3. The mean value of the permeability coefficients was 2.92 x 10^{-3} cm h^{-1} with a standard error of 2.78 x 10^{-4}.

4.3.2 Effect of Penetration Enhancers on the Permeation of Propranolol HCl through Hairless Mouse Skin

4.3.2.1 Sodium Dodecyl Sulphate (SDDS) as an Ion-Pairing Agent

4.3.2.1.1 Determination of the Apparent Solubility Product of the Complex Between SDDS and Propranolol HCl

The changes in apparent absorbance and conductivity with changes in the concentration of SDDS for the system with a propranolol HCl concentration of 7.96 x 10^{-4}M are shown in figure 4.8. The appearance of turbidity (end-point) was associated with a change in the slope of the conductivity curve. The two other systems in double distilled water showed similar behaviour with the appearance of turbidity in the solutions associated with a change in the slope of the conductivity curve. This appearance of an insoluble phase may be described by the solubility product of the ion-pair complex. The

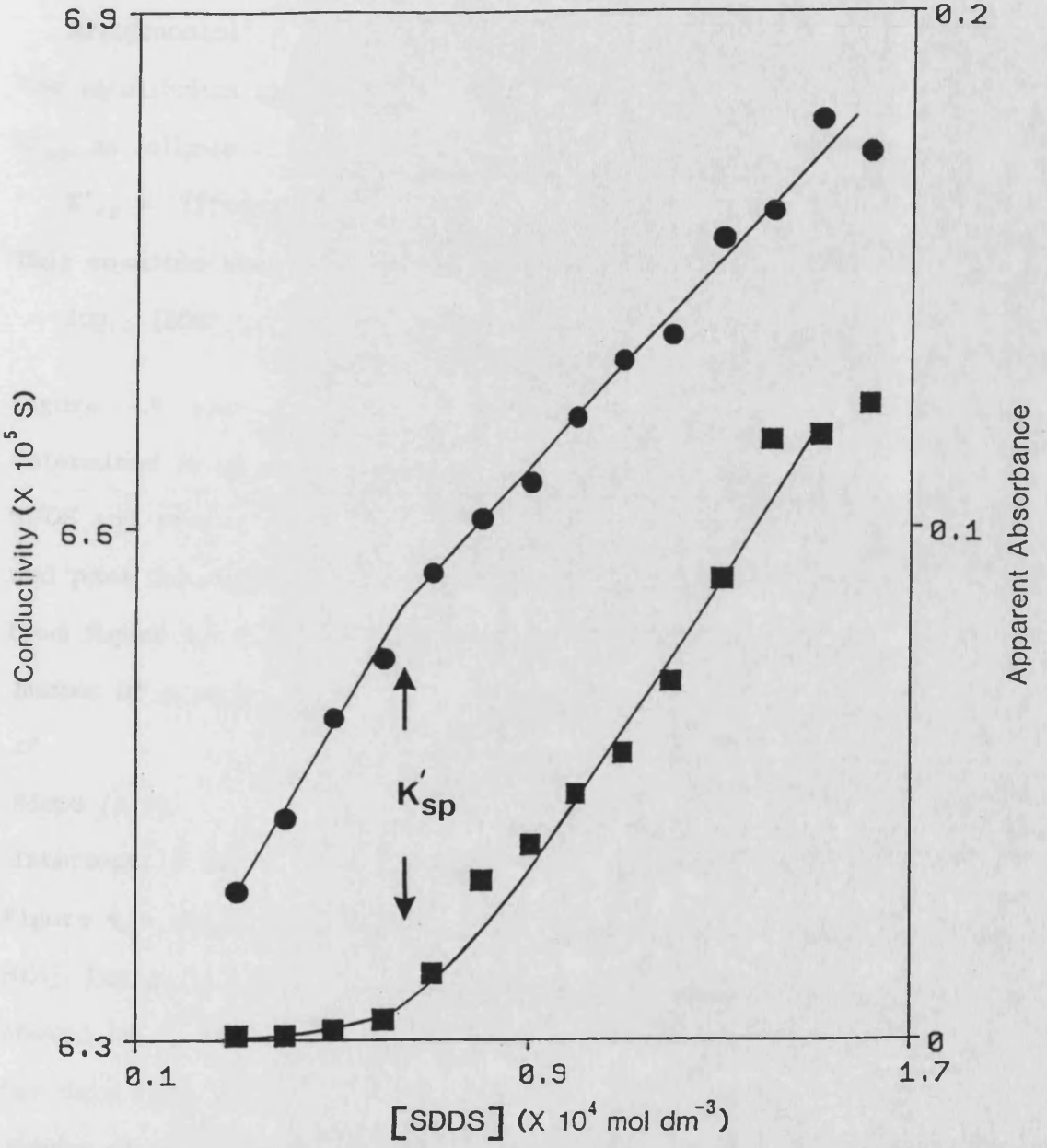


Figure 4.8. Changes in conductivity (●) and apparent absorbance (■) with SDDS concentration in the presence of 7.96 x 10^{-4}M propranolol HCl in double distilled water at 30°C.

equilibrium between the insoluble phase and the ions in solution may be written as follows :

$$x\text{Propranolol}^{+} + y\text{DDS}^{-} \rightleftharpoons \text{Propranolol}_x\text{DDS}_{y\,(\text{insoluble})}$$

The equilibrium may be defined by the apparent solubility product, K'_{sp}, as follows :

$$K'_{sp} = [\text{Propranolol}^{+}_{(aq)}]^{x} . [\text{DDS}^{-}_{(aq)}]^{y}$$

This equation may be rearranged to give the following :

$$\log_{10} [\text{DDS}^{-}_{(aq)}] = \frac{1}{y} \log_{10} K'_{sp} - \frac{x}{y} \log_{10} [\text{Propranolol}^{+}_{(aq)}]$$

Figure 4.9 shows a plot of [Propranolol HCl] versus $[SDDS]^{-1}$ determined from the complexation end-point. For a 1:1 complex between SDDS and propranolol HCl this plot should be linear with slope K'_{sp} and pass through the origin. Linear regression analysis of the data from figure 4.9 yielded the following statistics :

Number of points	:	3
r^2	:	0.9911
Slope (S.D)	:	5.30×10^{-8} (5.02×10^{-9})
Intercept (S.D)	:	-2.83×10^{-5} (5.50×10^{-5})

Figure 4.10 shows a double log plot of [SDDS] versus [Propranolol HCl]. For a 1:1 complex between SDDS and propranolol HCl this plot should be linear with a slope of unity. Linear regression analysis of the data from figure 4.10 yielded the following statistics :

Number of points	:	3
r^2	:	0.9893
Slope (S.D)	:	-0.868 (9.04×10^{-2})
Intercept (S.D)	:	-6.87 (0.305)

The results indicate a 1:1 stoichiometry for the complex between propranolol HCl and SDDS with an apparent solubility product at 30°C of 5.30×10^{-8} $mol^2\ dm^{-6}$.

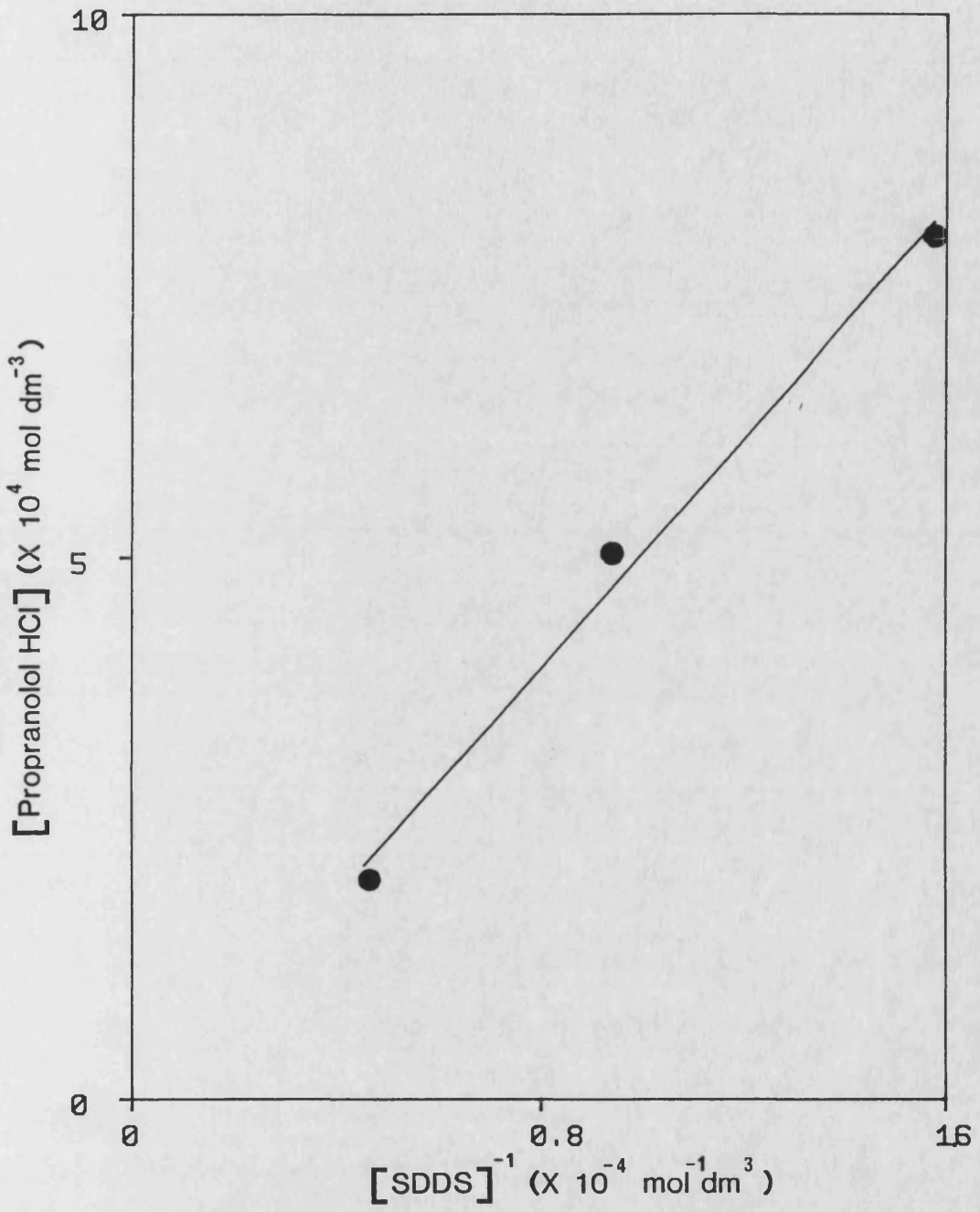


Figure 4.9. Relationship between propranolol HCl concentration and the reciprocal of SDDS concentration at complexation end point at 30°C.

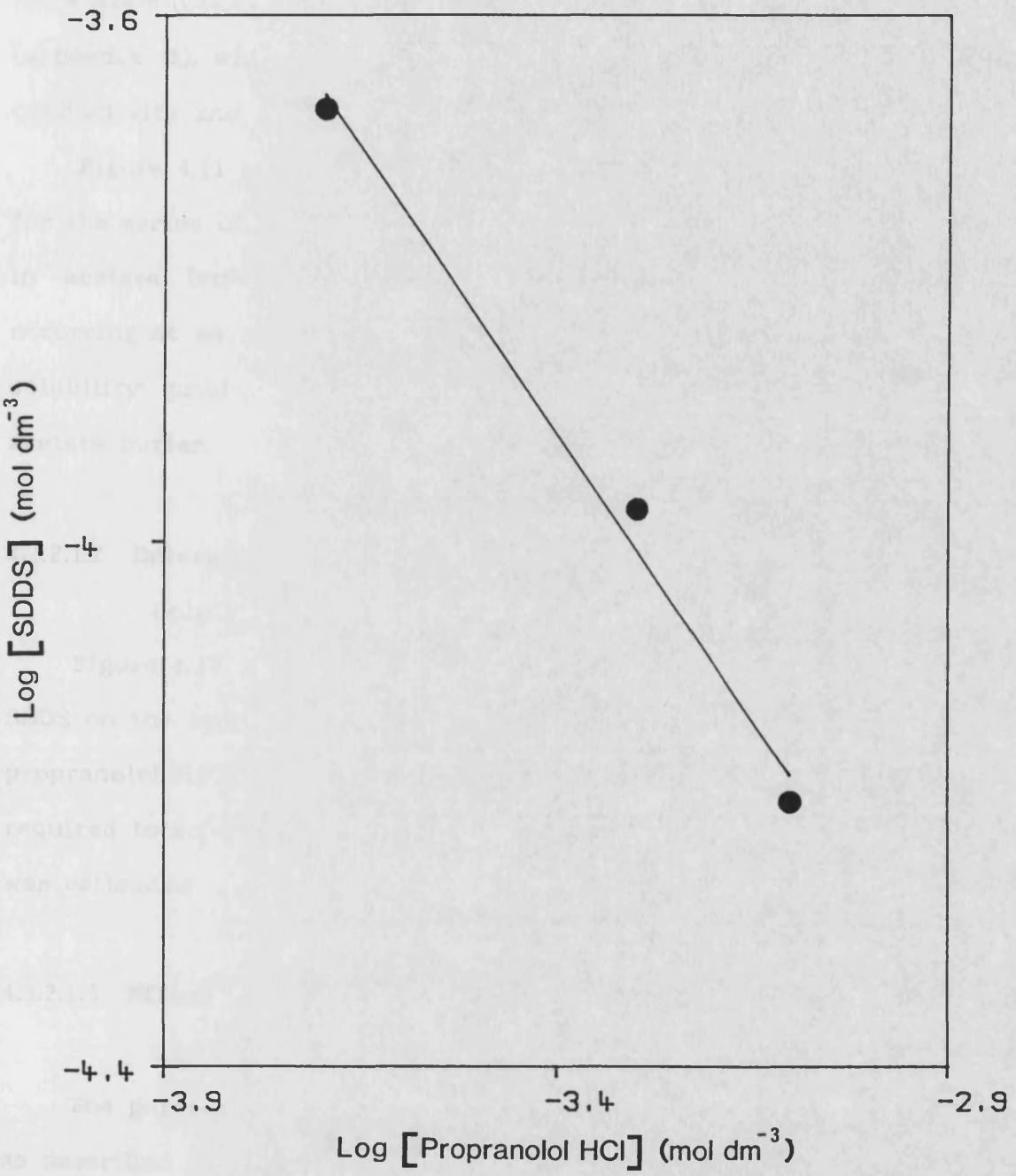


Figure 4.10. Relationship between the logarithm of SDDS concentration at complexation end-point and the logarithm of propranolol HCl concentration at 30°C.

The NMR spectrum of the anhydrous ion-pair complex in deuterated chloroform ($CDCl_3$) showed the complex to have a 1:1 stoichiometry (appendix 5), which is in agreement with the results obtained from conductivity and turbidity experiments.

Figure 4.11 shows a plot of apparent absorbance versus [SDDS] for the series of solutions containing 7.98 x 10^{-4}M propranolol HCl in acetate buffer. The complexation end-point was estimated as occurring at an SDDS concentration of 1.01 x 10^{-4}M, and the apparent solubility product estimated as 8.08 x 10^{-8} mol^2 dm^{-6} at 30°C in acetate buffer.

4.3.2.1.2 Determination of the Concentration of SDDS Required to Solubilise the Complex between SDDS and Propranolol HCl

Figure 4.12 shows the effect of increasing the concentration of SDDS on the apparent absorbance of solutions containing 8.45 x 10^{-3}M propranolol HCl in acetate buffer at 30°C. The concentration of SDDS required to solubilise the complex between SDDS and propranolol HCl was estimated as 2.69 x 10^{-2}M.

4.3.2.1.3 Effect of SDDS on the Permeation of Propranolol HCl through Hairless Mouse Skin

The permeability coefficients of propranolol HCl were determined as described in section 4.3.1.3, figure 4.13 shows the cumulative amount of propranolol HCl in the receptor compartment versus time and shows the effect of SDDS. The permeability coefficient of propranolol HCl in the presence of 9.4 x 10^{-6}M SDDS in acetate buffer was 5.26 x 10^{-3} cm h^{-1} with a standard error of 2.09 x 10^{-3}. This is a small but statistically non-significant ($P<0.05$) increase from the

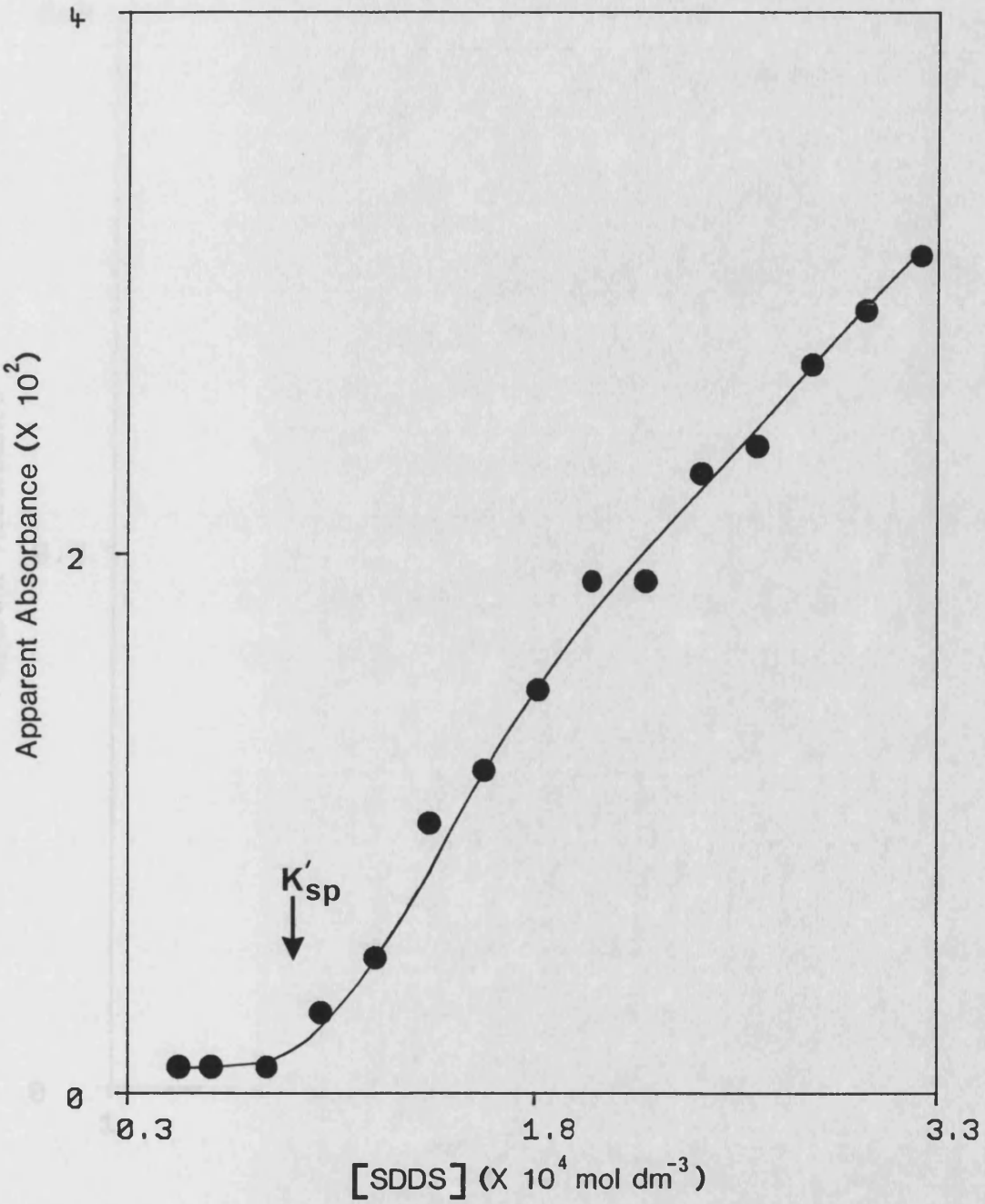


Figure 4.11. Change in apparent absorbance with SDDS concentration in the presence of 7.98 x 10^{-4}M propranolol HCl in acetate buffer at 30°C.

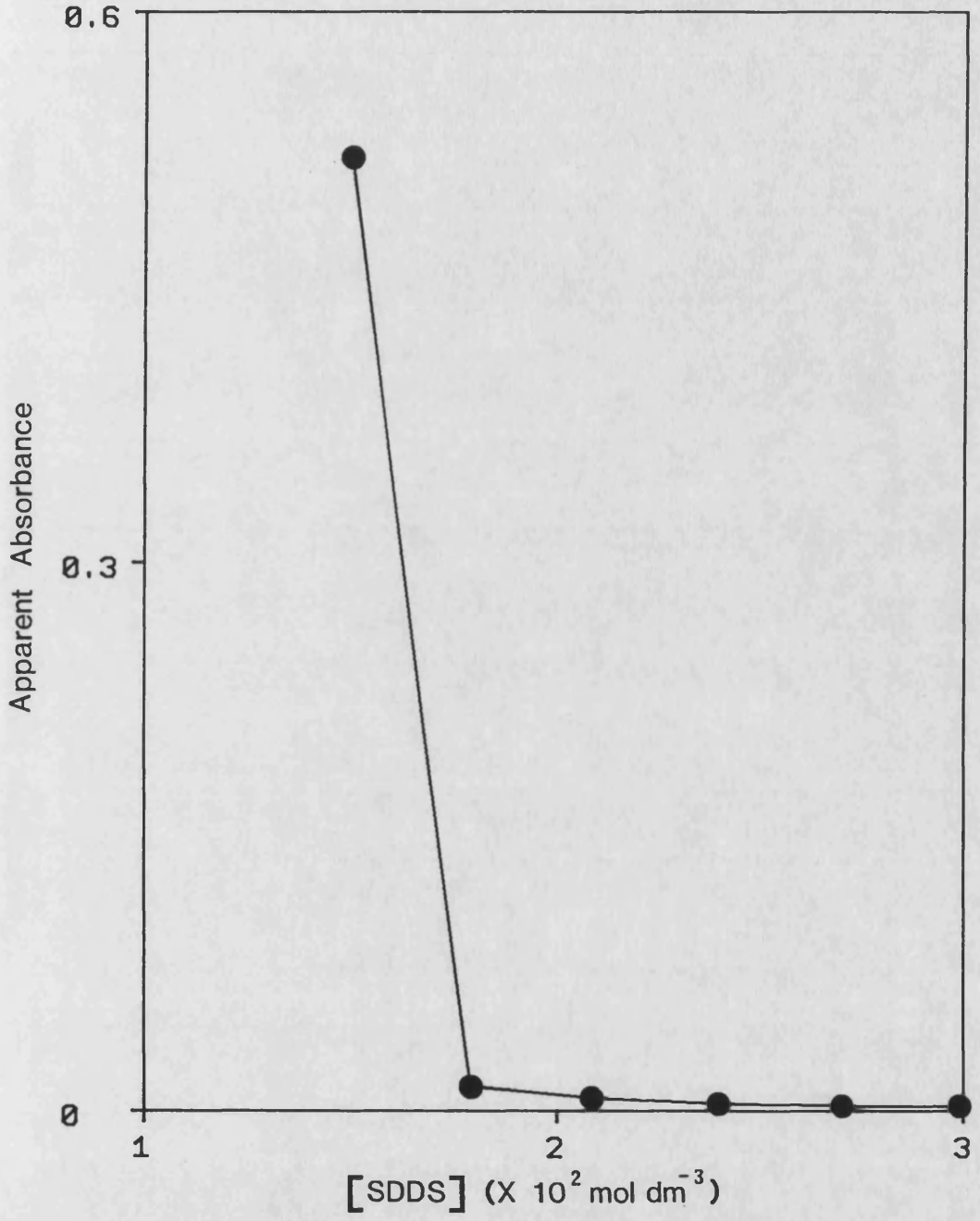


Figure 4.12. Change in apparent absorbance with SDDS concentration in the presence of 8.45 x 10^{-3}M propranolol HCl in acetate buffer at 30°C.

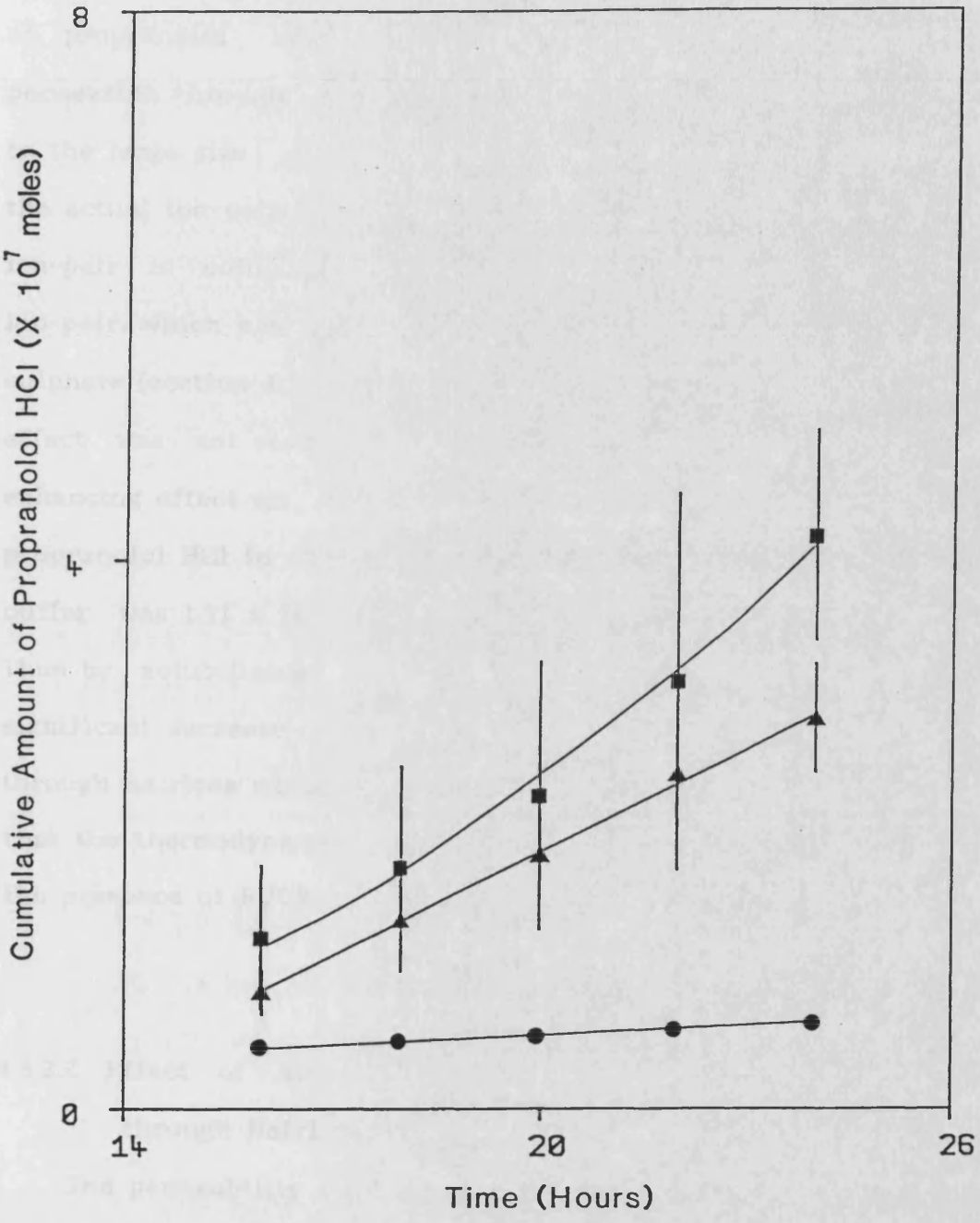


Figure 4.13. Relationship between cumulative amount of propranolol HCl in the receptor compartment versus time for a donor concentration of 8.4 mM propranolol HCl in the presence of a) 9.40 x 10^{-6}M SDDS in acetate buffer (—■—), b) acetate buffer (—▲—), c) 2.69 x 10^{-2}M SDDS in acetate buffer (—●—).

permeability coefficient determined in buffer alone. The ion-pairing of propranolol HCl was thus not successful in enhancing the permeation through hairless mouse skin. This is probably partly due to the large size of the ion-pair, but mainly due to the fact that the actual ion-pair concentration is very low. The concentration of ion-pair in solution is limited by the solubility product of the ion-pair, which has been shown to be low for propranolol dodecyl sulphate (section 4.3.2.1.1). It is perhaps surprising that a greater effect was not seen since surfactants such as SDDS have a direct enhancing effect on the skin [26]. The permeability coefficient of propranolol HCl in the presence of 2.69 x 10^{-2}M SDDS in acetate buffer was 1.71 x 10^{-4} cm h^{-1} with a standard error of 6.91 x 10^{-6}. Thus by solubilising the complex between SDDS and propranolol HCl a significant decrease ($P<0.05$) in the permeability of propranolol HCl through hairless mouse skin occurs. This may be explained by the fact that the thermodynamic activity of propranolol HCl is decreased in the presence of SDDS micelles.

4.3.2.2 Effect of Azone on the Permeation of Propranolol HCl through Hairless Mouse Skin

The permeability coefficients of propranolol HCl were determined as described in section 4.3.1.3 and are shown in table 4.2. Figure 4.14 is a plot of the cumulative amount of propranolol HCl in the receptor compartment versus time and shows the effect of the various formulations. The values of the permeability coefficients, together with those determined from sections 4.3.1.3 and 4.3.2.1 are compared in figure 4.15. The results indicate that neither Azone nor Tween 20

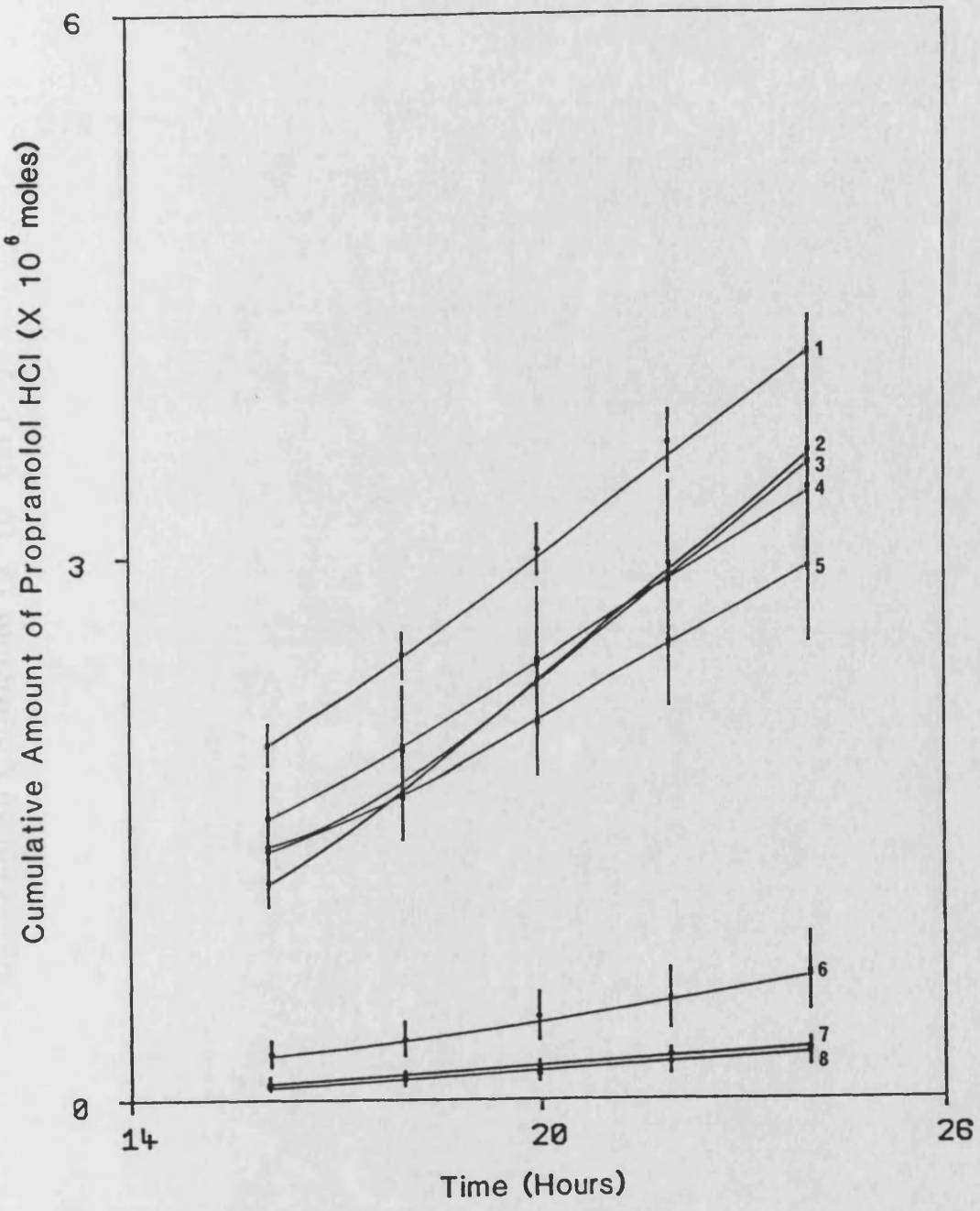


Figure 4.14. Relationship between cumulative amount of propranolol HCl in the receptor compartment versus time for a donor concentration of 8.4 mM propranolol HCl in the presence of i) acetate buffer (7), ii) 0.11% Tween 20 in acetate buffer (8), iii) 2% Azone in acetate buffer (6), iv) 0.11% Tween 20 in acetate buffer plus the following concentrations of Azone : 1% (3), 2% (2), 3% (4), 5% (1), 10% (5).

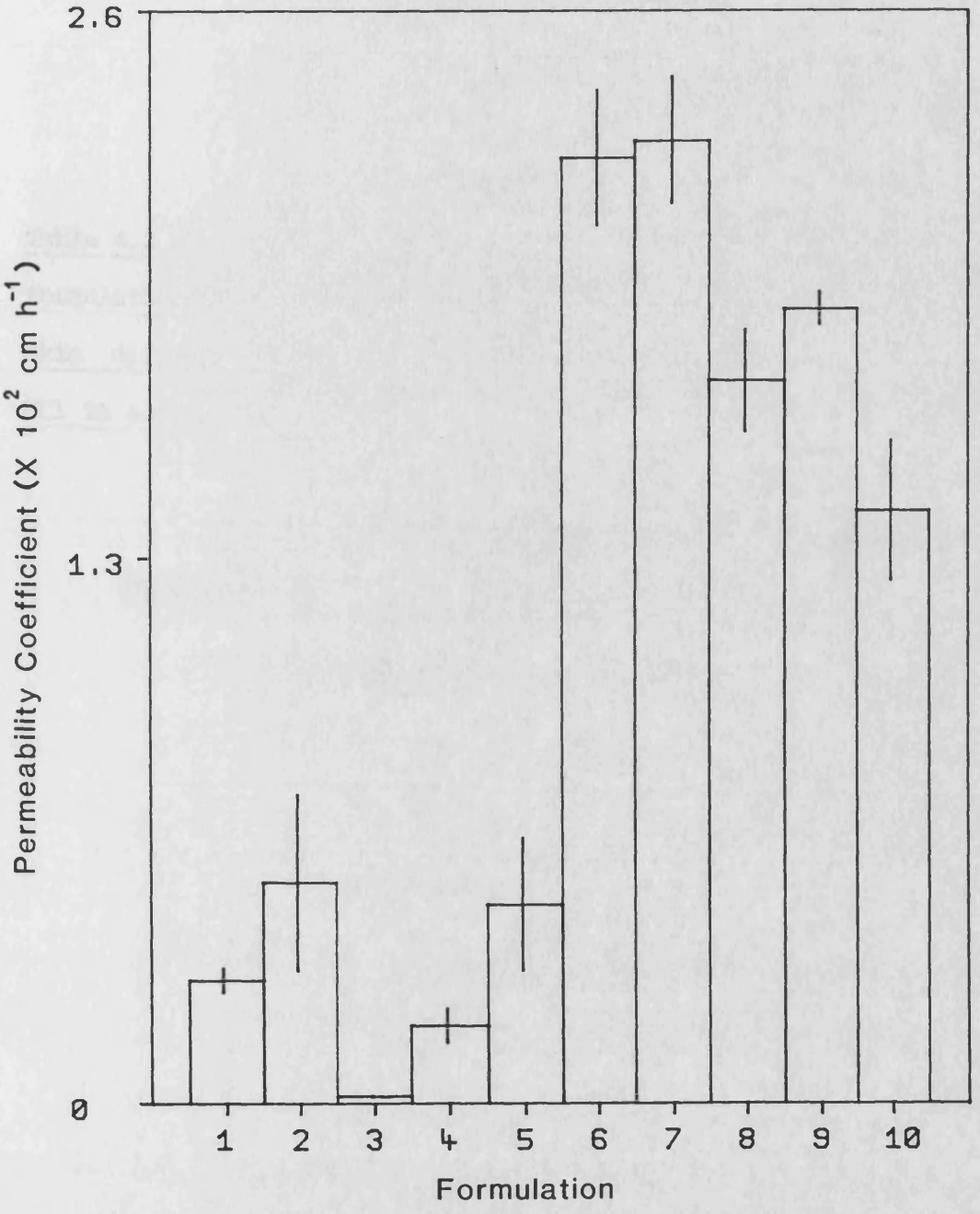


Figure 4.15. Effect of various formulations on the permeability coefficient of propranolol HCl through hairless mouse skin (see table 4.3 for key to formulations).

Table 4.3 Key to figure 4.15 showing the nature of the various formulations of propranolol HCl added to the donor compartment in the skin diffusion cell (each formulation is based on 8.4 mM propranolol HCl in acetate buffer).

Formulation Number	Formulation
1	-
2	9.40×10^{-6}M SDDS
3	2.69×10^{-2}M SDDS
4	0.11% Tween 20
5	2% Azone
6	1% Azone + 0.11% Tween 20
7	2% Azone + 0.11% Tween 20
8	3% Azone + 0.11% Tween 20
9	4% Azone + 0.11% Tween 20
10	5% Azone + 0.11% Tween 20

alone cause any significant increase ($P<0.05$) in the transport of propranolol HCl across hairless mouse skin. It can however be seen that when Azone is applied as an emulsion, enhancement in the transport of propranolol HCl does occur, and a significant increase ($P<0.05$) in the permeability coefficient is found. An emulsion of propranolol HCl and 2% Azone in buffer (with Tween 20) is found to have a permeability coefficient approximately 7.8 times greater than propranolol HCl in buffer alone. The data indicates that an emulsion of 1% Azone is as effective in enhancing the transport of propranolol HCl in comparison with higher concentrations of Azone, with a decrease in enhancer activity occurring in the presence of 3, 5 and 10% Azone, which may be due to changes in the thermodynamic activity of propranolol HCl in the donor phase at higher concentrations of Azone.

Table 4.2 Effect of Azone on the transport of propranolol HCl through hairless mouse skin.

Formulation	Permeability Coefficient ($cm\ h^{-1}$)	Standard Error
A	4.73×10^{-3}	1.58×10^{-3}
B	1.84×10^{-3}	3.84×10^{-4}
C	2.25×10^{-2}	1.62×10^{-3}
D	2.29×10^{-2}	1.50×10^{-3}
E	1.72×10^{-2}	1.21×10^{-3}
F	1.89×10^{-2}	3.80×10^{-4}
G	1.41×10^{-2}	1.66×10^{-3}

4.3.3 In Vitro Model of a Transdermal Electrophoretic Device

Figure 4.16 shows a continuous plot of the change in measured concentration of propranolol HCl in the receptor compartment versus time. The control experiment, together with the experiment with buffer alone in the cathode compartment indicate that the hairless mouse skin yields u.v. absorbing materials into solution which interfered with the assay. Therefore the true concentration of propranolol HCl at any given time can not be accurately determined by the analytical method employed. The control experiment indicates that the amount of u.v. absorbing material leached by the skin increased slowly and continuously after an initial burst; the release of these materials being unaffected by the application of an electrophoretic current of 2 mA. Thus the plots may give an indication of the changes in the concentration of propranolol HCl in the receptor compartment. The results indicate that Azone has an enhancing effect on the transport of propranolol HCl into the receptor compartment and that the application of an electric current across the electrodes increased the delivery rate of propranolol HCl into the receptor compartment after a lag period. The increase in the delivery rate produced by an electrophoretic current appears to be irreversible. This may be explained by the fact that the skin is the rate limiting step for drug delivery into the receptor compartment. During application of an electrophoretic current the concentration of propranolol HCl in the cathode compartment probably increases, thus the driving force of diffusion-controlled transport across the skin would be expected to increase, which is reflected by an increase in the delivery rate of propranolol HCl into the receptor compartment after a short lag time. A possible way of decreasing drug delivery

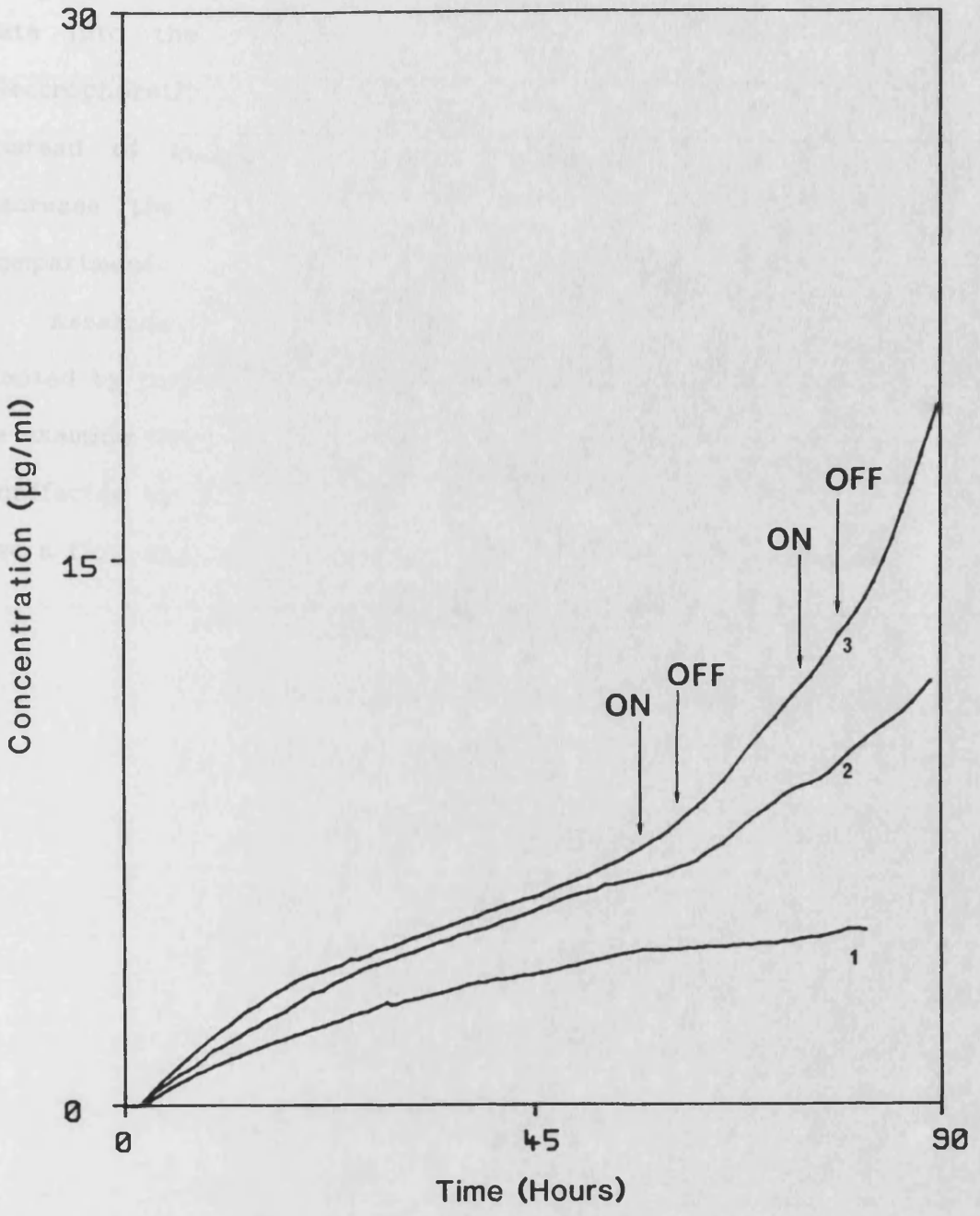


Figure 4.16. Electrophoretically modulated transdermal delivery of propranolol HCl showing the change in apparent propranolol HCl concentration with time in the receptor compartment for (1) control, (2) acetate buffer in cathode compartment, (3) 1% Azone + 0.11% Tween 20 in cathode compartment.

Note : On = electrophoretic current switched on.

Off = electrophoretic current switched off.

rate into the receptor compartment could be to apply an electrophoretic current with the polarity of the electrodes reversed instead of simply switching off the current, in an attempt to decrease the concentration of propranolol HCl in the cathode compartment.

Assessment of the performance of the model investigated is limited by the analytical technique, however it would be useful to re-examine the system with another continual assay method that is unaffected by interfering substances. One possible method would be to use a flow through scintillation counter rather than a u.v. detector.

Conclusions

Conclusions

High pressure liquid chromatography of propranolol HCl (PHC) in aqueous solution indicated that PHC is stable in solution for at least one month at 25°C and at a pH below 5.3.

Homogeneous crosslinked PHEMA was prepared by chemically initiated solution polymerization, whereas uniform polymerization by gamma irradiation in the conditions available was unsuccessful. The equilibrium water content and the swelling behaviour of PHEMA when stored in water was found to be comparable with previously reported studies. However in acetate buffer PHEMA showed complex swelling behaviour. Scanning electron microscopy of PHEMA did not reveal any porous ultrastructure at a magnification of X5000 - the polymer surface appeared to be smooth and regular.

PHC was found to interact with PHEMA in solution, this interaction could only be partly explained by the presence of negatively charged binding sites shown to be present in PHEMA, the presence of which were thought to be related to initial monomer purity.

PHC showed anomalous diffusion behaviour through PHEMA with respect to the effect of altering crosslinker content. A decrease in crosslinker content resulted in an increase in the diffusion coefficient which was consistent with a change in diffusion mechanism from a predominantly partition mechanism to a pore mechanism, however the changes in diffusion coefficient at lower crosslinker content were anomalous with respect to the diffusion

behaviour of previously reported solutes. Methylating PHEMA resulted in a small but significant decrease in the diffusion coefficient of propranolol HCl through PHEMA at low crosslinker content, but no difference was found at higher crosslinker contents.

In the model electrophoretic system examined, significant degradation of PHC occurred at a current of 15 mA. The presence of platinum black on the platinum electrodes caused degradation at lower currents in the range of 0 to 2.4 mA. However, by using uncoated platinum electrodes and currents in this range no detectable degradation of PHC was found during the course of experiments. No localized heating effects were found in the PHEMA films during electrophoresis. Constant current electrophoresis in the current range 0 to 2.4 mA produced predictable and reproducible increases in the delivery rate of PHC in the model system. The effect produced by a current was dependent on the crosslinker content of the PHEMA film. Several factors affected the delivery rate of PHC during electrophoresis, such as ionic strength, temperature and reservoir PHC concentration. Reversal of electrode polarity resulted in a reduction of the basal drug delivery rate due to diffusion. Application of an electrophoretic current during the diffusion lag time was found to produce a different effect to that found when electrophoresis was carried out during steady state diffusion. Feedback experiments demonstrated that control over the delivery rate of PHC from the model electrophoretic system could be achieved by means of feedback control.

The effect of the permeation enhancers sodium dodecyl sulphate (SDDS) and Azone on the permeation of PHC through hairless mouse skin was investigated. SDDS caused an insignificant increase on the permeation of PHC through hairless mouse skin when present at a concentration which did not exceed the apparent solubility product between PHC and SDDS. SDDS present at a concentration above that necessary to solubilise the complex coacervate between SDDS and PHC caused a significant decrease in the permeability coefficient of PHC through hairless mouse skin. The stoichiometry of the complex between SDDS and PHC was found to be 1:1. Azone significantly enhanced the permeation of PHC through hairless mouse skin.

A model of a transdermal electrophoretic drug delivery device was investigated. However, the u.v. assay used was not entirely satisfactory due to significant leaching of interfering substances. Nevertheless the model did indicate that some control over drug delivery could be achieved, although results from the same model indicated that controlled drug delivery across the skin may be restricted by long lag times.

The use of an electrophoretically modulated drug delivery device is considered to be a feasible method of providing true controlled drug delivery. Significant control over drug delivery is possible using low constant currents. The degree of control that this type of device potentially offers would make it suitable for use as an implant device, possibly acting as a closed-loop drug delivery device.

Appendices

APPENDIX 1

Measurement of Diffusion Coefficients from Permeation through Membranes [286-290].

The mathematical theory of passive diffusion in an isotropic medium is based on Fick's laws of diffusion. Diffusion is the tendency of a solute to distribute itself uniformly over the space available to it due to the random movement of molecules and is a probabilistic process which is spontaneous, irreversible and thermodynamically favourable. Fick's first law of diffusion states that the rate of transfer of a diffusing substance across a plane of unit area, known as flux (J), is proportional to the concentration gradient across the plane (dC/dx) as stated below :

$$J = -D\frac{dC}{dx} \qquad \text{(eq. 1)}$$

where D, the diffusion coefficient, is the constant of proportionality. The negative sign indicates that the flux is in the direction of decreasing concentration. The diffusion coefficient may be considered a constant at a given temperature, however in some situations it may be concentration dependent and is best regarded as a mean for the concentration under consideration.

Fick's second law of diffusion states that the rate of change in concentration in a volume element within a diffusional field (dC/dt) is proportional to the rate of change in concentration at that point in the field (d^2C/dx^2) as stated below :

$$\frac{dC}{dt} = D\frac{d^2C}{dx^2} \qquad \text{(eq. 2)}$$

Fick's second law is a general equation which has many complex solutions depending on the boundary conditions being considered. The case commonly encountered is the simple zero-order flux situation.

This solution is applicable where we have a membrane (eg. polymer or skin) of thickness h separating two compartments, where the concentration gradient across the membrane may be considered constant and the receptor compartment is kept at essentially sink conditions. The defined boundary conditions are that unidirectional diffusional flow occurs beginning at x=0, the high concentration side of the membrane, and continues toward the other membrane surface where x=h. The concentration of the diffusant substance at x=0 is a constant (C_o) at all values of time (t), also the concentration of the diffusant (C) at x=h is zero at all values of time. The model also assumes that at t=0, for all values of x greater than zero, C=0. The diffusion coefficient is also assumed to be invariable. The solution of Fick's second law for these given boundary conditions is usually expressed in terms of the cumulative mass of diffusant (M) which passes through unit area of the membrane in time t, as shown below :

$$M = \frac{DC_o t}{h} - \frac{hC_o}{6} - \frac{2hC_o}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\exp\left(\frac{-Dn^2\pi^2 t}{h^2}\right) \qquad \text{(eq. 3)}$$

As t approaches infinity the third term in the equation tends to zero and the equation can be reduced to the straight line expression shown below :

$$M = \frac{DC_o}{h}\left(t - \frac{h^2}{6D}\right) \qquad \text{(eq. 4)}$$

Equation 4 applies once steady state diffusion has been attained where dC/dt is zero at all values of x within the membrane. The steady state flux of the diffusant (dM/dt) may be obtained by differentiating equation 4 as shown below :

$$\frac{dM}{dt} = \frac{DC_o}{h} \qquad \text{(eq. 5)}$$

If the steady state line from equation 4 is extrapolated to the time

axis, one may obtain the lag time (t_L) as shown below :

$$t_L = \frac{h^2}{6D} \qquad \text{(eq. 6)}$$

Steady state diffusion is not assumed to have been attained until a time of at least 2.7 times the lag time. It can therefore be seen that from a permeation experiment the diffusion coefficient may be estimated provided the membrane thickness is known.

In most experiments the actual value C_o, the concentration of diffusant in the first layer of the membrane, is not known. The value actually measured is the concentration in the donor phase bathing the membrane, C', which may be related to C_o by the partition coefficient (K) as follows :

$$C_o = C'K \qquad \text{(eq. 7)}$$

Thus substituting equation 7 into equation 4 yields the following :

$$M = \frac{DKC'}{h}\left(t - \frac{h^2}{6D}\right) \qquad \text{(eq. 8)}$$

If the volume (V) of the receptor compartment is known then equation 8 may be written in terms of the measured concentration of diffusant in the receptor compartment (C_t) as follows :

$$C_t = \frac{DKC'}{hV}\left(t - \frac{h^2}{6D}\right) \qquad \text{(eq. 9)}$$

A plot of equation 9 is sometimes termed a Barrer plot.

APPENDIX 2

Measurement of pH and Preparation of Buffers

Calibration of the pH meters (Radiometer PHM64 research pH meter, V.A. Howe and Co. Ltd., London, U.K., Philips PW 9421 pH meter, Pye Unicam Ltd., Cambridge, U.K.) was carried out using the following primary standard buffer solutions [271] :

Buffer	pH at 25°C
0.05M potassium hydrogen phthalate	4.005
0.01M disodium tetraborate	9.185
0.025M potassium dihydrogen orthophosphate and 0.025M disodium hydrogen orthophosphate	6.858

The standard buffers were prepared by dissolving appropriate amounts of the buffer salts in freshly distilled water. Prior to buffer preparation all salts except for the disodium tetraborate, were dried over phosphorous pentoxide in a vacuum oven at 80°C to remove any traces of water.

The buffer salts used in this work were all based on the following systems unless otherwise stated :

pH at 25°C	Buffer*
4.48	Walpole's acetate
3.03	McIlvaine's citric acid-phosphate
10.05	Delory and King's carbonate-bicarbonate

*[291]

The pH of each of the buffers was measured after equilibrating samples of buffer in a water bath held at 25°C. All buffers were stored at 4°C in the dark and discarded after four weeks.

APPENDIX 3

U/V Detector Calibrating Program

```
 10MODE135:CLEAR:@%=&2000A
 20*FX213,250
 30*FX214,3
 40*FX16,1
 50VDU 23;8202;0;0;0;
 60DIM Z(12),Y1(12),X1(12),AB(12)
 70PRINTTAB(2,11)"_____"
 80PRINTTAB(2,12)"* Detector Data Collecting Program *"
 90PRINTTAB(2,13)"_____1_____"
100TIME=0
110REPEAT
120UNTIL TIME>324
130CLS
140PRINTTAB(2,8)"CONTINUE (C) OR CALIBRATE AGAIN (R)"
150Z%=GET
160IF Z%=67 THEN PROCCHECK
170IF Z%<>82 THEN150
180CLS
190PRINTTAB(2,5)"CALIBRATION OF DETECTOR"
200PRINTTAB(2)"_____"
210PRINTTAB(2,12)"How Many Points Do You Want?"
220INPUTTAB(2,14)"? "O%
230CLS
240PRINTTAB(2,12)" Enter Maximum Concentration Value"'
250INPUT"          "Y1(2)
260Y1(1)=0
270X1(1)=0:X1(2)=36650
280CLS
290PRINTTAB(2,6)"Choose Type Of Run"
300PRINTTAB(2)"_____"
310PRINTTAB(2,10)"(1) Auto Data Capture"
320PRINTTAB(2,12)"(2) Auto Data Capture With Absorbances"
330X%=GET-48
340IFX%<>1 AND X%<>2 THEN 330
350PROCCALIB
360CLS:@%=&1050A
370PRINT'"" ADVAL(1)  CONC      "
380PRINT" _______ _______"'
390FOR P%= 3 TO O%+2
400PRINT X1(P%) Y1(P%)
410NEXT
420PRINT'""PRESS ANY KEY TO CONTINUE"
430D=GET
440IF X%=1 THEN470
450PROCREG
460PROCBL
470FORP%=3 TO O%+2
480SUMX=SUMX+X1(P%):SUMXX=SUMXX+X1(P%)*X1(P%)
490SUMY=SUMY+Y1(P%):SUMYY=SUMYY+Y1(P%)*Y1(P%)
500SUMXY=SUMXY+Y1(P%)*X1(P%)
510NEXT
520A=SUMXX-(SUMX*SUMX)/O%
```

```
530B=SUMYY-(SUMY*SUMY)/O%
540C=SUMXY-(SUMY*SUMX/O%)
550 CC=C/(SQR(A*B))
560SL=C/A
570IN=(SUMY-SL*SUMX)/O%
580FOR P%=3 TO O%+2
590Z(P%)=SL*X1(P%)+IN
600SUMDSQ=SUMDSQ+((Z(P%)-Y1(P%))^2)
610NEXT
620DENOM=(O%*A)
630IF SUMDSQ=0 THENSDS=0:SDI=0:GOTO670
640SDS=(O%*SUMDSQ/((O%-2)*DENOM))^.5
650SDI=(SUMXX*SUMDSQ/((O%-2)*DENOM))^.5
660CLS
670PRINT'"    CALIBRATION ANALYSIS"'
680@%=10:PRINT"        No. of Points     ";O%
690@%=&20508:PRINT:PRINT"        Corr. Coeff.     ";CC
700@%=&1050A:PRINT:PRINT"        Slope Coeff      ";SL'
710PRINT"        S.D. of Slope     ";SDS'
720PRINT"        Intercept         ";IN'
730PRINT"        S.D. of Intercept ";SDI''
740PRINT"PRESS ANY KEY TO CONTINUE"
750E%=GET
760MODE128
770PROCGRAPH
780MODE135
790PRINTTAB(2,8)"Have You inserted disc?"
800J%=GET
810IF J%<>89 THEN800
820B=OPENOUT "SLIN"
830PRINT B,O%,SL,IN,SLA,INA,CC,SDS,SDI,A1,B1
840CLOSE B
850CHAIN"GIL"
860END
870DEF PROCCALIB
880FOR H%=3 TO O%+2
890CLS
900PRINTTAB(2,12)"What is Drug Concentration ";H%-2
910INPUTTAB(2,15)"?   "Y1(H%)
920IF Y1(H%)=0 THEN 900
930CLS
940@%=&1050A
950PRINTTAB(2,2)"Drug Concentration= ";Y1(H%)
960@%=&2000A
970PRINTTAB(2,4)"(Press S When Detector is Stable)"
980PRINTTAB(2,6)"(Must Not Exceed 2290)"'
990PRINT"Detector Output= "'
1000FORV%=1 TO 14
1010WT%=TIME
1020IF TIME< WT%+1 THEN 1020
1030PRINTTAB(13,9+V%)ADVAL(1)/16
1040NEXT
1050C%=INKEY(30)
1060IFC%<>83 THEN 940
1070AV%=0
1080FORI%=1TO 1000
```

```
1090WT%=TIME
1100IF TIME < WT%+1 THEN 1100
1110AV%=AV%+ ADVAL(1)
1120NEXT
1130X1(H%)=AV%/1000
1140IFX%=2 THEN PROCABS
1150NEXT
1160IFX%=2 THEN1180
1170SLA=0:INA=0
1180ENDPROC
1190DEF PROCABS
1200CLS
1210INPUTTAB(2,6)"What is Absorbance  ?  "AB(H%)
1220CLS
1230ENDPROC
1240DEF PROCREG
1250FORP%=3TOO%+2
1260SX=SX+X1(P%):SXX=SXX+X1(P%)*X1(P%)
1270SY=SY+AB(P%):SYY=SYY+AB(P%)*AB(P%)
1280SXY=SXY+AB(P%)*X1(P%)
1290NEXT
1300AA=SXX-(SX*SX)/O%
1310CA=SXY-(SY*SX/O%)
1320SLA=CA/AA
1330INA=(SY-SLA*SX)/O%
1340ENDPROC
1350DEF PROCBL
1360FORP%=3TOO%+2
1370SX1=SX1+Y1(P%):SXX1=SXX1+Y1(P%)*Y1(P%)
1380SY1=SY1+AB(P%):SYY1=SYY1+AB(P%)*AB(P%)
1390SXY1=SXY1+AB(P%)*Y1(P%)
1400NEXT
1410AA1=SXX1-(SX1*SX1)/O%
1420CA1=SXY1-(SY1*SX1/O%)
1430A1=CA1/AA1
1440B1=(SY1-A1*SX1)/O%
1450ENDPROC
1460DEF PROCGRAPH
1470CLG:@%=&1030A:VDU5
1480MOVE250,1000:DRAW250,125:DRAW1250,125:MOVE275,125:DRAW275,100:
MOVE1225,125:DRAW1225,100:MOVE250,150:DRAW225,150:MOVE250,950:DRA
W225,950
1490MOVE50,958:PRINTY1(2):MOVE50,158:PRINTY1(1):MOVE190,100:PRINTX1
(1):MOVE1124,100:PRINTX1(2)
1500MOVE50,550:PRINT"CONC.":MOVE650,100:PRINT"DETECTOR OUTPUT"
1510MOVE275,(X1(1)*SL+IN-Y1(1))/(Y1(2)-Y1(1))*800+150:DRAW1225,(X1(
2)*SL+IN-Y1(1))/(Y1(2)-Y1(1))*800+150
1520FOR P=3 TO O%+2
1530MOVE(X1(P)-X1(1))/(X1(2)-X1(1))*950+267,(Y1(P)-Y1(1))/(Y1(2)-Y1
(1))*800+166:PRINT"x"
1540NEXT
1550MOVE600,50:PRINT"PRESS ANY KEY TO CONTINUE"
1560J%=GET
1570VDU4
1580ENDPROC
1590DEF PROCCHECK
```

```
1600CLS
1610PRINTTAB(2,8)"Have You inserted disc?"
1620J%=GET
1630IF J%<>89 THEN1620
1640CHAIN"GIL"
1650ENDPROC
```

Data Collecting Program for Diffusion and Electrophoresis Experiments

```
  10REM A D'Emanuele
  20REMUniversity of Bath
  30MODE135:CLEAR
  40*FX229,7
  50*FX11,0
  60*FX213,250
  70*FX214,3
  80*FX16,1
  90?&FDFA=0
 100?&FDF9=0
 110VDU23;8202;0;0;0;
 120DIMY(350,6)
 130DIMZ(20,2)
 140H%=0
 150DP%=0
 160W%=0
 170a%=0
 180Q%=0
 190b%=1
 200N%=0
 210C%=1
 220C=OPENIN"SLIN"
 230INPUTC,O%,c,d,e,f,g,h,i,j,k
 240CLOSEC
 250PRINT''"    CALIBRATION ANALYSIS"''
 260@%=10:PRINT"        No. of Points     ";O%
 270@%=&20508:PRINT:PRINT"        Corr. Coeff.      ";g
 280@%=&1050A:PRINT:PRINT"        Slope Coeff       ";c'"
("";j;")"'
 290PRINT"        S.D. of Slope     ";h'
 300PRINT"        Intercept         ";d'"        (";k;")"'
 310PRINT"        S.D. of Intercept ";i''''
 320PRINT"CONTINUE (C) OR CALIBRATE AGAIN (R)"
 330Z%=GET
 340IFZ%>95THENZ%=Z%-32
 350IFZ%=67THEN380
 360IFZ%<>82THEN330
 370CHAIN"GIL1"
 380MODE135:VDU23;8202;0;0;0;
 390PRINTTAB(4,6)"What is the Power Supply Scale ?"
 400PRINTTAB(4,10)"(1) 0-25  mA"
 410PRINTTAB(4,12)"(2) 0-2.5 mA"
 420INPUTTAB(4,15)"Enter 1 or 2  "l
 430IFl<>1ANDl<>2THEN380
 440IFl=1THENl=10ELSEl=100
 450CLS
 460IFl=10THEN PROCm ELSE PROCn
```

```
470PRINTTAB(4,15)"Press any Key to Continue"
480Z%=GET
490MODE131
500VDU23;8202;0;0;0;
510PRINTTAB(12,2)"Electrophoresis/Diffusion Cell Programs (210ml
Cell)"
520PRINTTAB(11,3)"-----------------------------------------
--------"
530PRINTTAB(25,5)"Choose Type of Experiment"
540PRINTTAB(24,6)"-------------------------"
550PRINTTAB(4,10)"Keyboard Control of Power Supply at any Time.
(Check PROCv)          (1)"
560PRINTTAB(4,13)"Power Supply Turned On and Off at Fixed Times.
                (2)"
570PRINTTAB(4,16)"Feedback Control (Resulting in Fixed Flux from
a Given Start Time).  (3)"
580PRINTTAB(4,19)"Fixed Release Profile (Set PROCv, run program
again)                  (4)"
590PRINTTAB(4,20)"choosing option (1) )"
600INPUTTAB(25,23)"Choose Option  "L%
610IFL%<1ORL%>3THEN490
620MODE135
630PRINTTAB(2,3)TIME$
640PRINTTAB(2,5)"What is the Title?"
650INPUTTAB(2,7)T$
660IFT$=""THEN650
670PRINTTAB(2,9)"Enter Sample Interval (s)"
680INPUTTAB(2,11)o%
690IFo%=0THEN680
700PRINTTAB(2,13)"Enter Maximum Concentration %w/v"
710INPUTTAB(2,15)p
720IFp=0THEN710
730MODE131
740VDU23;8202;0;0;0;
750ONL%PROCq,PROCr,PROCs,PROCt
760VDU15
770PRINTTAB(2,21)"PRESS SPACE WHEN READY TO BEGIN "
780IFGET<>32THEN780
790CLS
800D$=TIME$
810T$=T$+" "+D$
820TIME=0
830Y%=0
840E%=0
850u%=0
860IFTIME<o%*u%*100THEN950
870PROCv
880PROCw
890MODE128:VDU23;8202;0;0;0;
900PROCx
910u%=u%+1
920IFY%=1THEN950
930PRINTTAB(10,31)"PRESS M FOR MENU"
940IFY%=350THEN1730
950IFL%=1THEN980
960IFL%=2ORL%=3THENPROCy ELSE980
```

```
 970IFN%=1THEN1380
 980PROCv
 990PROCw
1000IFTIME<W%THEN1020
1010PROCz
1020IFE%=0THEN1070
1030PROCaa
1040M%=INKEY(1)
1050IFM%>95THENM%=M%-32
1060IFM%<>77THEN860
1070IFQ%=1THEN1210
1080MODE135:VDU23;8202;0;0;0;
1090PRINTTAB(15,1)"   MENU"
1100PRINTTAB(14,2)"_____________"
1110PRINTTAB(4,4)"Change Current              (1)"
1120PRINTTAB(4,6)"Change Polarity             (2)"
1130PRINTTAB(4,8)"Read Current and Voltage    (3)"
1140PRINTTAB(4,10)"Read Conc. and Absorbance  (4)"
1150PRINTTAB(4,12)"Read Drug Delivery Rate    (5)"
1160PRINTTAB(4,14)"Collect Extra Data         (6)"
1170PRINTTAB(4,16)"Plot Graph                 (7)"
1180PRINTTAB(4,18)"Stop Run                   (8)"
1190PRINTTAB(2,22)"Press Selection Number"
1200Q%=1
1210E%=0:M%=0:J%=58
1220J%=INKEY(1)
1230IFJ%>95THENJ%=J%-32
1240K%=J%-48
1250ONK%PROCab,PROCa,PROCac,PROCad,PROCae ELSE 1260
1260IFK%<>6THEN1610
1270@%=10
1280CLS
1290PRINTTAB(4,4)"Number of Points Available = ";349-Y%
1300PRINTTAB(4,6)"How Many Points Do You Want ?"
1310INPUTTAB(4,8)Z%
1320IFZ%<1ORZ%>(349-Y%)THEN1600
1330PRINTTAB(4,10)"What Time Intervals Do You Want     Samples
Taken in Seconds.  (Must Be     Greater Than 60)"
1340INPUTTAB(4,15)af%
1350VDU7
1360IFaf%<60THEN1330
1370af%=af%*100
1380F%=0
1390G%=0
1400MODE128
1410VDU23;8202;0;0;0;
1420ag%=TIME
1430REPEAT
1440IFL%=1THEN1460
1450IFL%=2 OR L%=3 THENPROCy
1460PROCw
1470IFTIME<W%THEN1490
1480PROCz
1490IFTIME<(ag%+G%)THEN1530ELSEPROCx
1500PRINTTAB(10,31)"Press S to Stop"
1510F%=F%+1
```

```
1520G%=G%+af%
1530ah%=INKEY(1)
1540PROCaa
1550IFah%>95THENah%=ah%-32
1560UNTILF%=Z%ORah%=83ORY%=350
1570u%=u%+INT(af%*F%/o%/100)
1580CLS
1590PRINTTAB(10,31)"PRESS M FOR MENU"
1600Q%=0:E%=0
1610IFK%<>7THEN1650
1620MODE128:VDU23;8202;0;0;0;
1630PROCai
1640PRINTTAB(10,31)"PRESS M FOR MENU"
1650IFK%=8THEN1660ELSE860
1660CLS
1670Q%=0
1680PRINTTAB(2,10)"Are You Sure You Want to Stop"
1690K%=INKEY(500)
1700IFK%>95THENK%=K%-32
1710IFK%=89THEN1720ELSE860
1720MODE131
1730PROCaj
1740MODE135
1750CLS
1760PRINTTAB(5,10)"PROGRAM FINISHED"
1770END
1780DEFPROCx
1790PRINTTAB(15,16)"SAMPLING DETECTOR"
1800PROCak
1810PROCal
1820IFY%=1THEN1840
1830PROCai
1840ENDPROC
1850DEFPROCak
1860am%=0
1870FORI%=1TO1000
1880an%=TIME
1890am%=am%+ADVAL(1)
1900IFTIME<an%+1THEN1900
1910NEXT
1920am%=am%/1000
1930ao=am%*e+f
1940ap=am%*c+d
1950ENDPROC
1960DEFPROCal
1970Y%=Y%+1
1980Y(Y%,1)=INT(TIME/100)
1990Y(Y%,2)=ap
2000Y(Y%,3)=ao
2010Y(Y%,4)=?&FDFC/10
2020Y(Y%,5)=?&FDFB/1
2030IFH%<10THEN2050
2040Y(Y%,6)=ae
2050ENDPROC
2060DEFPROCw
2070IFC%=1THEN2120
```

```
2080PROCDP
2090V%=?&FDFB
2100IFV%>w%THENPROCaq
2110IFV%<w%THENPROCar
2120IFC%=0THEN2140
2130?&FDFA=0
2140ENDPROC
2150DEFPROCaq
2160?&FDFA=P%
2170A%=INKEY(50)
2180IF?&FDFB<=w%THEN2240
2190REPEAT
2200P%=P%-1
2210?&FDFA=P%
2220A%=INKEY(50)
2230UNTIL?&FDFB<=w%
2240ENDPROC
2250DEFPROCar
2260?&FDFA=P%
2270A%=INKEY(50)
2280IF?&FDFB>=w%ORP%=250THEN2340
2290REPEAT
2300P%=P%+1
2310?&FDFA=P%
2320A%=INKEY(50)
2330UNTIL?&FDFB>=w%ORP%=250
2340ENDPROC
2350DEFPROCy
2360IFN%=2THEN2400
2370IFN%=1THEN2390
2380IFTIME>=as AND TIME<at THEN PROCau
2390IFTIME>=at THEN PROCav
2400ENDPROC
2410DEFPROCz
2430W%=W%+30000
2440H%=H%+1
2450PROCak
2460Z(H%,1)=INT(TIME/100)
2470Z(H%,2)=ap
2480IFH%<10THEN2650
2490aw=0:ax=0:ay=0:az=0:ba=0
2500FORI%=(H%-9)TOH%
2510aw=aw+Z(I%,1):ay=ay+Z(I%,1)^2
2520ax=ax+Z(I%,2):az=az+Z(I%,2)^2
2530ba=ba+Z(I%,1)*Z(I%,2)
2540NEXT
2550bb=ay-(aw*aw)/10
2560bc=az-(ax*ax)/10
2570bd=ba-(ax*aw)/10
2580IFbb=0THEN2630
2590be=bd/bb
2600ae=be*7.56E6
2610IF(bc*bb)<=0THEN2630
2620bg=bd/(SQR(bb*bc))
2630IFH%=20THENPROCbh
2640IFL%=3THENPROCbi
```

```
2650ENDPROC
2660DEFPROCbi
2670IFN%<>1THEN2690
2680IF ae<s THEN PROCbj ELSE PROCbk
2690ENDPROC
2700DEFPROCbj
2710w%=w%+INT(100*((s-ae)/0.44483))
2720IF w%>250 THEN w%=250
2730PROCw
2740ENDPROC
2750DEFPROCbk
2760w%=w%-INT(100*((ae-s)/0.44483))
2770IF w%<0 THEN w%=0
2780PROCw
2790ENDPROC
2800DEFPROCbh
2810FORI%=1TO10
2820Z(I%,1)=Z(I%+10,1)
2830Z(I%,2)=Z(I%+10,2)
2840NEXT
2850FORI%=11TO20
2860Z(I%,1)=0:Z(I%,2)=0
2870NEXT
2880H%=10
2890ENDPROC
2900DEFPROCai
2910CLG:@%=&1030A:VDU5
2920MOVE250,950:DRAW250,125:DRAW1250,125:MOVE275,125:DRAW275,100:
MOVE1225,125:DRAW1225,100:MOVE250,150:DRAW225,150:MOVE250,900:DRAW
225,900
2930MOVE50,908:PRINTp:MOVE180,158:PRINT"0":MOVE265,95:PRINT"0":
MOVE1124,95:PRINTY(Y%,1)/3600
2940MOVE50,550:PRINT"CONC.%W/V":MOVE650,100:PRINT"TIME(HOURS)"
2950MOVE(Y(1,1)/3600-0)/(Y(Y%,1)/3600-0)*950+277,
(Y(1,2)-0)/(p-0)*750+150
2960FORP=2TOY%
2970DRAW(Y(P,1)/3600-0)/(Y(Y%,1)/3600-0)*950+277,
(Y(P,2)-0)/(p-0)*750+150
2980NEXT
2990FORP=1TOY%
3000MOVE(Y(P,1)/3600-0)/(Y(Y%,1)/3600-0)*950+269,
(Y(P,2)-0)/(p-0)*750+166:PRINT"x"
3010NEXT
3020VDU4
3030VDU7
3040E%=1
3050Q%=0
3060ENDPROC
3070DEFPROCab
3080Q%=0
3090CLS
3100IFC%=0THEN3150
3110PRINTTAB(2,9)"Do you want the Power Supply turned on?"
3120D%=INKEY(400)
3130IFD%>95THEND%=D%-32
3140IFD%=89THENC%=0ELSEGOTO3250
```

```
3150PRINTTAB(2,9)"Enter New Current in mA                    "
3160INPUTTAB(2,13)bm
3170IFl=10THEN3190
3180IF bm>2.5 THEN3090
3190IF bm>25 THEN3090
3200w%=bm*l
3210IFw%=0THENC%=1
3220P%=w%
3230?&FDFA=w%
3240PROCw
3250ENDPROC
3260DEFPROCac
3270Q%=0
3280CLS
3290IFC%=1THEN3380
3300@%=&0002020A
3310PRINTTAB(2,3)"Time = ";INT(TIME/100);" Secs)"
3320PRINTTAB(2,5)"( ";TIME/360000;" Hours)"
3330@%=&0002010A
3340PRINTTAB(2,8)"Current = ";?&FDFB/l;" mA"
3350PRINTTAB(2,10)"Voltage = ";?&FDFC/10;" V"
3360PRINTTAB(2,18)"PRESS ANY KEY TO CONTINUE"
3370B%=INKEY(800)
3380ENDPROC
3390DEFPROCad
3400Q%=0
3410CLS
3420PRINTTAB(15,16)"SAMPLING DETECTOR"
3430PROCak
3440CLS
3450@%=&0002020A
3460PRINTTAB(2,3)"Time = ";INT(TIME/100);" Secs)"
3470PRINTTAB(2,5)"( ";TIME/360000;" Hours)"
3480@%=&0001040A
3490PRINTTAB(2,8)"Concentration = "ap
3500@%=&0002040A
3510PRINTTAB(2,10)"Absorbance = ";ao
3520PRINTTAB(2,18)"PRESS ANY KEY TO CONTINUE"
3530B%=INKEY(800)
3540ENDPROC
3550DEFPROCau
3560F%=0
3570G%=0
3580ag%=TIME
3590N%=1
3600C%=0
3610w%=bn
3620P%=w%
3630?&FDFA=w%
3640Z%=S%
3650af%=U%
3660ENDPROC
3670DEFPROCav
3680N%=2
3690C%=1
3700?&FDFA=0
```

```
3710ENDPROC
3720DEFPROCaj
3730CLS
3740@%=10
3750PRINTTAB(2,8)"Have you inserted Master Statistics Disc ?"
3760REPEAT
3770VDU7
3780R%=GET
3790IFR%>95THENR%=R%-32
3800UNTILR%=89
3810CLS
3820PRINTTAB(2,2)T$
3830PRINTTAB(2,5)"What is the title of worksheet"
3840INPUTTAB(2,7)T$
3850IFT$=""THEN3840
3860PRINTTAB(2,10)"What is worksheet name"
3870INPUTTAB(2,12)bo$
3880IFbo$=""THEN3870
3890PRINTTAB(2,15)"What is data file name"
3900INPUTTAB(2,17)bp$
3910IFbp$=""THEN3900
3920*MOUNT
3930*DIR GIL
3940OSCLI("SPOOL "+bp$)
3950PRINT"CRE @";bo$;";COL 12 ";Y%+10;";CON 10;STR 10"
3960PRINTT$
3970@%=&0001050A
3980PRINT"READ X1-X6"
3990FORB%=1TOY%
4000PRINTY(B%,1);Y(B%,2);Y(B%,3);Y(B%,4);Y(B%,5);Y(B%,6)
4010NEXT
4020PRINT
4030PRINT"NAM X1 'TIME X2 'CONC X3 'AU X4 'V X5 'ImA X6 'DDR "
4040PRINT"LOC X1-X6"
4050PRINT
4060*SPOOL
4070ENDPROC
4080DEFPROCs
4090@%=10
4100CLS
4110INPUTTAB(2,8)"What DDR  do you require for Steady State Line
(mg/h) ?        "s
4120INPUTTAB(2,12)"What Time Do You Wish to Start Feedback Control
(Hours) ?  "as
4130as=as*360000
4140INPUTTAB(2,16)"What Time Do You Wish to Stop  Feedback Control
(Hours) ?  "at
4150at=at*360000
4160U%=30000
4170S%=INT(at-as)/30000+3
4180bn=INT(100*((s-0.12967)/0.44483))
4190ENDPROC
4200DEFPROCae
4210CLS
4220IFH%<10THEN4340
4230@%=&0002020A
```

```
4240PRINTTAB(2,3)"Time = ";INT(TIME/100);" Secs)"
4250PRINTTAB(2,5)"( ";TIME/360000;" Hours)"
4260@%=&0001040A
4270PRINTTAB(2,8)"Slope = ";be;" (%w/v/s)"
4280@%=&0002040A
4290PRINTTAB(2,10)"DDR    = ";ae;" mg/h"
4300PRINTTAB(2,12)"r      = ";bg
4310PRINTTAB(2,15)"(Data Based on Ten Samples Over The     Last 30
Minutes)"
4320PRINTTAB(2,22)"PRESS ANY KEY TO CONTINUE"
4330B%=INKEY(800)
4340Q%=0
4350ENDPROC
4360DEFPROCq
4370CLS
4380PRINTTAB(2,17)"Do you want the Power Supply?"
4390J%=GET
4400IFJ%<>89ANDJ%<>78THEN4390
4410IFJ%=78THENC%=1ELSEC%=0
4420IFC%=1THEN4510
4430PRINTTAB(2,17)"Enter Initial Current mA      "
4440INPUTTAB(2,19)bm
4450IF1=10THEN4470
4460IFbm>2.5THEN4370
4470IFbm>25THEN4370
4480w%=bm*1
4490P%=w%
4500?&FDFA=w%
4510ENDPROC
4520DEFPROCr
4530INPUTTAB(2,9)"What Time Do you want the Power Supply Turned On
(Hours) ?  "as
4540INPUTTAB(2,13)"What Time Do you want the Power Supply Turned Off
(Hours) ?  "at
4550as=as*360000
4560at=at*360000
4570INPUTTAB(2,17)"Enter Current in mA  "bn
4580IF1=10THEN4600
4590IFbn>2.5THEN4570
4600IFbn>25THEN4570
4610bn=bn*1
4620S%=INT(at-as)/30000+3
4630U%=30000
4640ENDPROC
4650DEFPROCm
4660PRINTTAB(3,6)"Check That Power Supply is set to"
4670PRINTTAB(3,8)"25V and 25mA"
4680ENDPROC
4690DEFPROCn
4700PRINTTAB(3,6)"Check That Power Supply is set to "
4710PRINTTAB(3,8)"25V and 2.5mA"
4720ENDPROC
4730DEFPROCaa
4740@%=&0002030A
4750IFH%<10THEN4770
4760PRINTTAB(18,4)"DDR      = ";ae;" mg/h (r=";bg;")";"   "
```

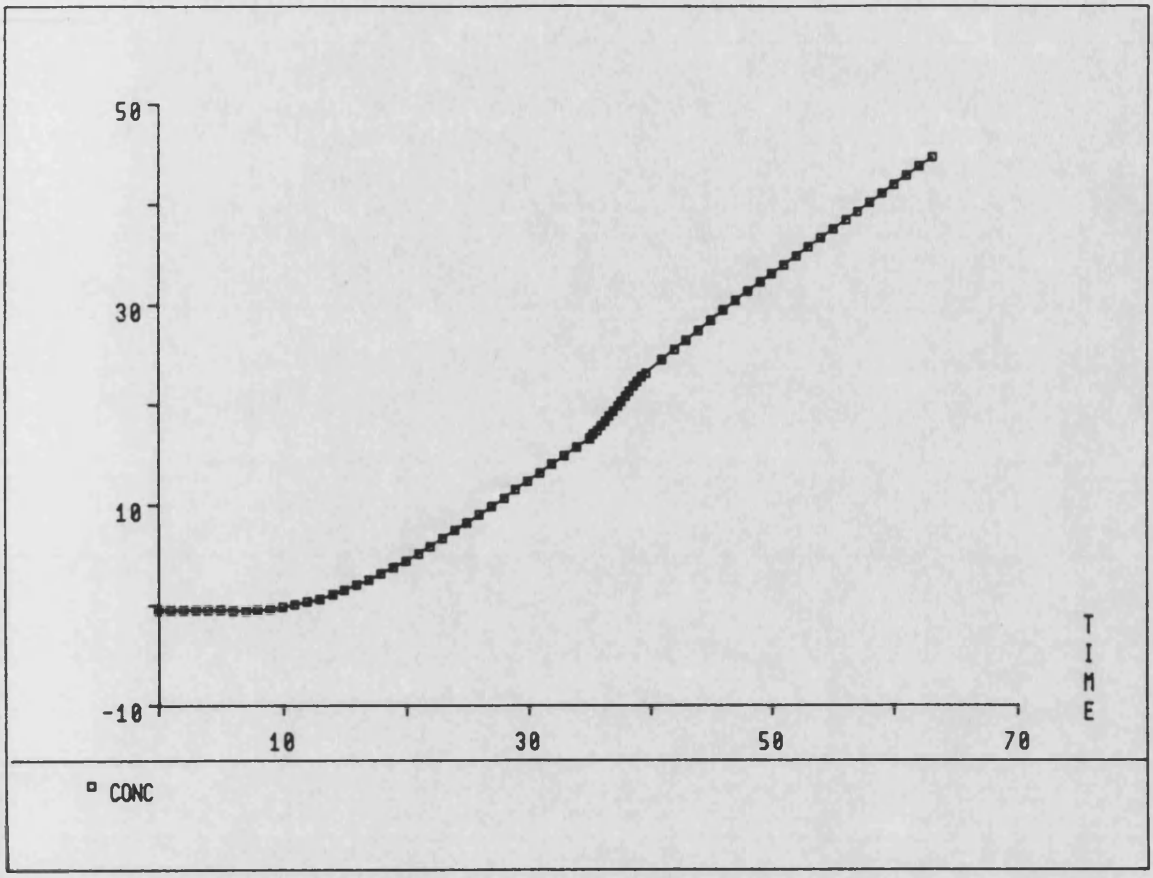
```
4770@%=&0002020A
4780IF C%=1 THEN4810
4790PRINTTAB(18,5)"Current = ";?&FDFB/1;" mA"
4800GOTO 4820
4810PRINTTAB(18,5)"                              "
4820PRINTTAB(18,3)"Time     = ";TIME/360000;" Hours"
4830ENDPROC
4840DEFPROCa
4850Q%=0
4860CLS
4870IFa%=1THEN4930
4880PRINTTAB(2,10)"Polarity is Currently Driving Flux, Do
    you Want to Change"
4890K%=INKEY(500)
4900IFK%>95THENK%=K%-32
4910IFK%=89THENa%=1ELSE4970
4920GOTO4970
4930PRINTTAB(2,10)"Polarity is Currently Opposing Flux,
    Do you Want to Change"
4940K%=INKEY(500)
4950IFK%>95THENK%=K%-32
4960IFK%=89THENa%=0ELSE4970
4970?&FDF9=a%
4980ENDPROC
4990DEFPROCv
5000REM ALTER ROUTINE FOR RELEASE PROFILE
5010REM GOTO ENDPROC (5450) IF NO PROFILE
5020REM ALTER a%,C%,P%,w%,?&FDF9
5030REM ALTER ?&FDFA,THEN GOTO ENDPROC
5040IFTIME<(57*360000)ORb%>1THEN5090
5050a%=0
5060?&FDF9=a%
5070C%=0:P%=200:w%=200:b%=2
5080?&FDFA=200
5090IFTIME<(61*360000)ORb%>2THEN5140
5100a%=0
5110?&FDF9=a%
5120C%=1:P%=0:w%=0:b%=3
5130?&FDFA=0
5140IFTIME<(75*360000)ORb%>3THEN5190
5150a%=0
5160?&FDF9=a%
5170C%=0:P%=200:w%=200:b%=4
5180?&FDFA=200
5190IFTIME<(79*360000)ORb%>4THEN5450
5200a%=0
5210?&FDF9=a%
5220C%=1:P%=0:w%=0:b%=5
5230?&FDFA=0
5240GOTO5450
5250IFTIME<(32*360000)ORb%>5THEN5300
5260a%=1
5270?&FDF9=a%
5280C%=0:P%=109:w%=109:b%=6
5290?&FDFA=109
```

```
5300IFTIME<(38*360000)ORb%>6THEN5450
5310C%=1:P%=0:w%=0:b%=7
5320?&FDFA=0
5330IFTIME<(64*360000)ORb%>7THEN5360
5340C%=0:P%=100:w%=100:b%=8
5350?&FDFA=100
5360IFTIME<(70*360000)ORb%>8THEN5390
5370C%=1:P%=0:w%=0:b%=9
5380?&FDFA=0
5390IFTIME<(77*360000)ORb%>9THEN5420
5400C%=0:P%=110:w%=110:b%=10
5410?&FDFA=110
5420IFTIME<(83*360000)ORb%>10THEN5450
5430C%=1:P%=0:w%=0:b%=11
5440?&FDFA=0
5450ENDPROC
5460DEF PROCDP
5470REM DELETE 5480 TO RUN ROUTINE
5480GOTO5550
5490IF DP%>TIME THEN 5550
5500DP%=TIME+6000
5510?&FDFA=0
5520A%=INKEY(1000)
5530?&FDFA=P%
5540A%=INKEY(50)
5550ENDPROC
```

APPENDIX 4

Controlled Transport of Propranolol HCl through Crosslinked PHEMA Films Using Constant Current Electrophoresis

The following pages contain examples of the concentration against time profiles obtained from electrophoresis experiments. The figures are screen dumps from the statistics package which was used to analyse the data (INSTAT). The examples demonstrate the effect of applying a constant current for a period of four hours. In all the graphs the units of concentration are µg/ml and the units of time are hours.



Experimental Conditions :

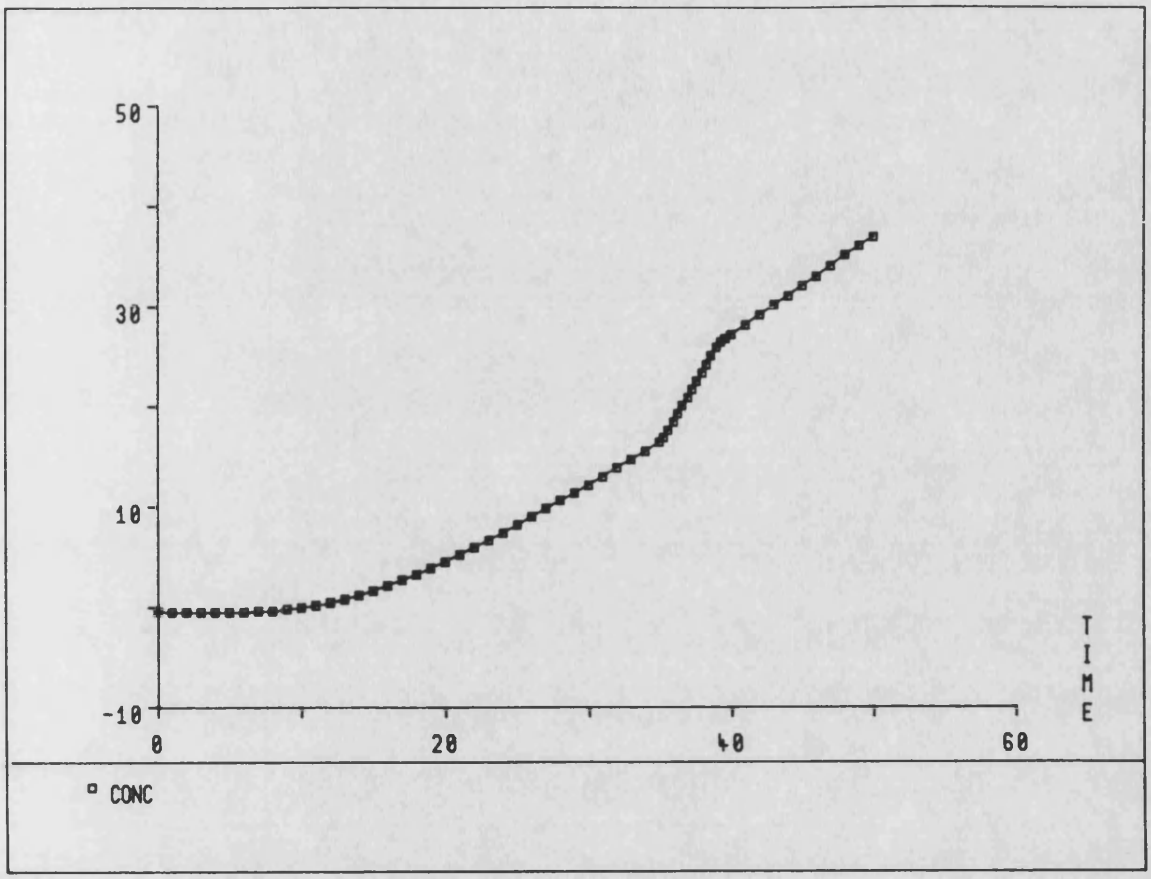
Applied Current of 0.40 mA between 35 and 39 Hours.

1%C PHEMA Film Thickness = 0.090 cm.

Initial Donor Propranolol HCl Concentration = 14.5 mM.

Temperature = 25°C.

Buffer Ionic Strength = 0.039.



Experimental Conditions :

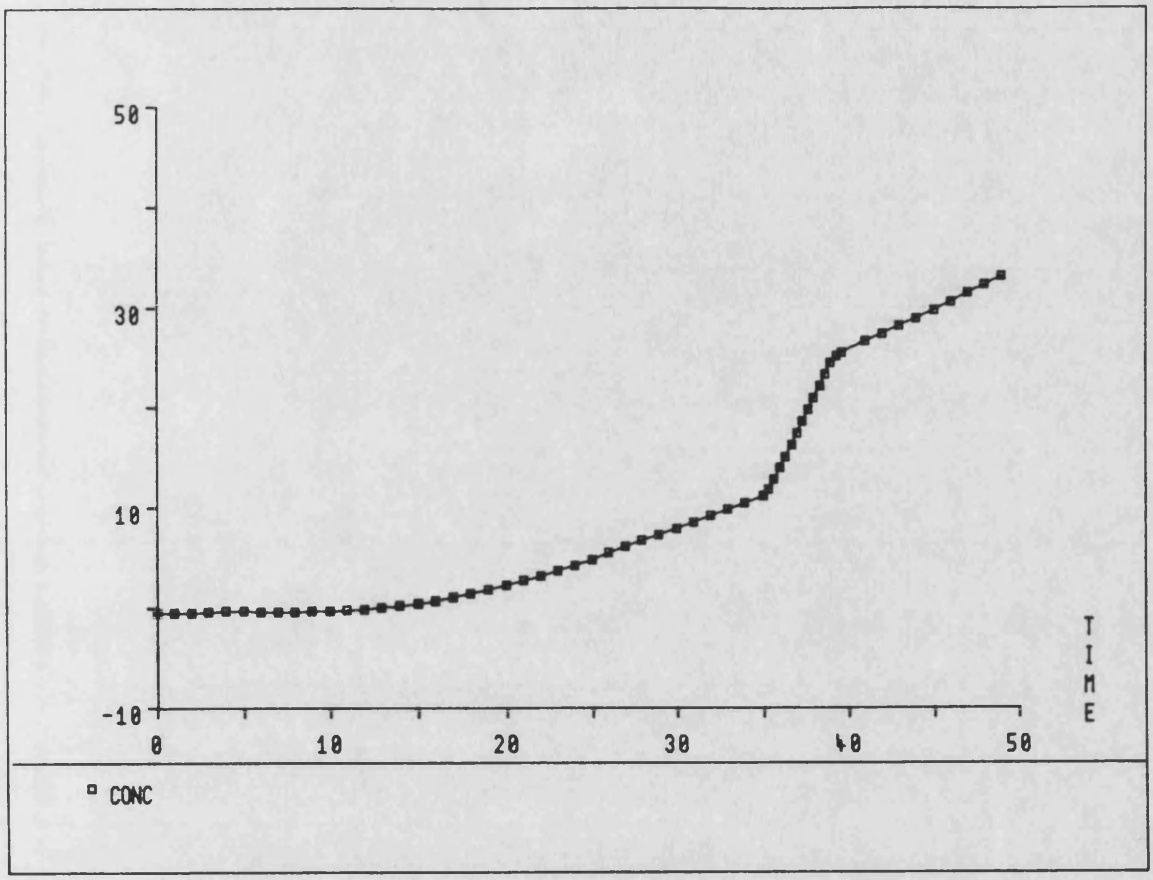
Applied Current of 0.90 mA between 35 and 39 Hours.

1%C PHEMA Film Thickness = 0.091 cm.

Initial Donor Propranolol HCl Concentration = 14.7 mM.

Temperature = 25°C.

Buffer Ionic Strength = 0.039.



Experimental Conditions :

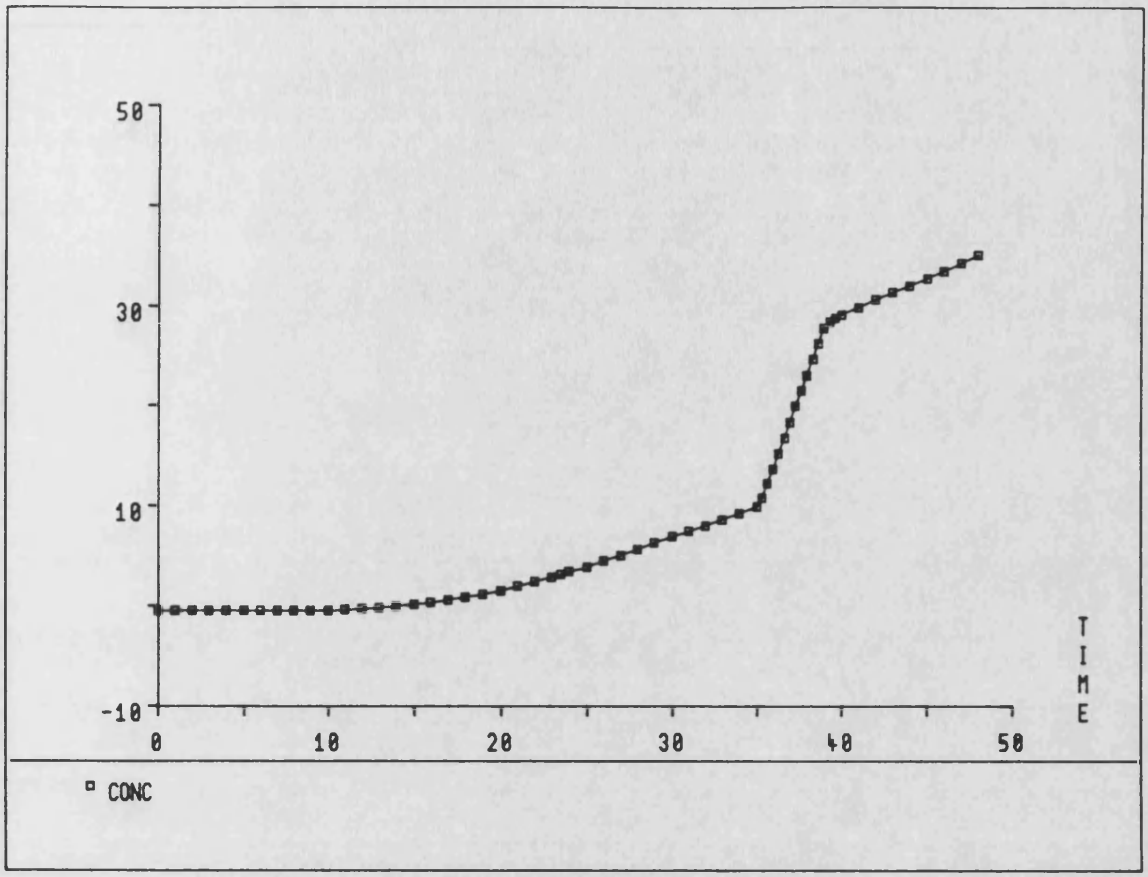
Applied Current of 1.40 mA between 35 and 39 Hours.

1%C PHEMA Film Thickness = 0.101 cm.

Initial Donor Propranolol HCl Concentration = 14.7 mM.

Temperature = 25°C.

Buffer Ionic Strength = 0.039.



Experimental Conditions :

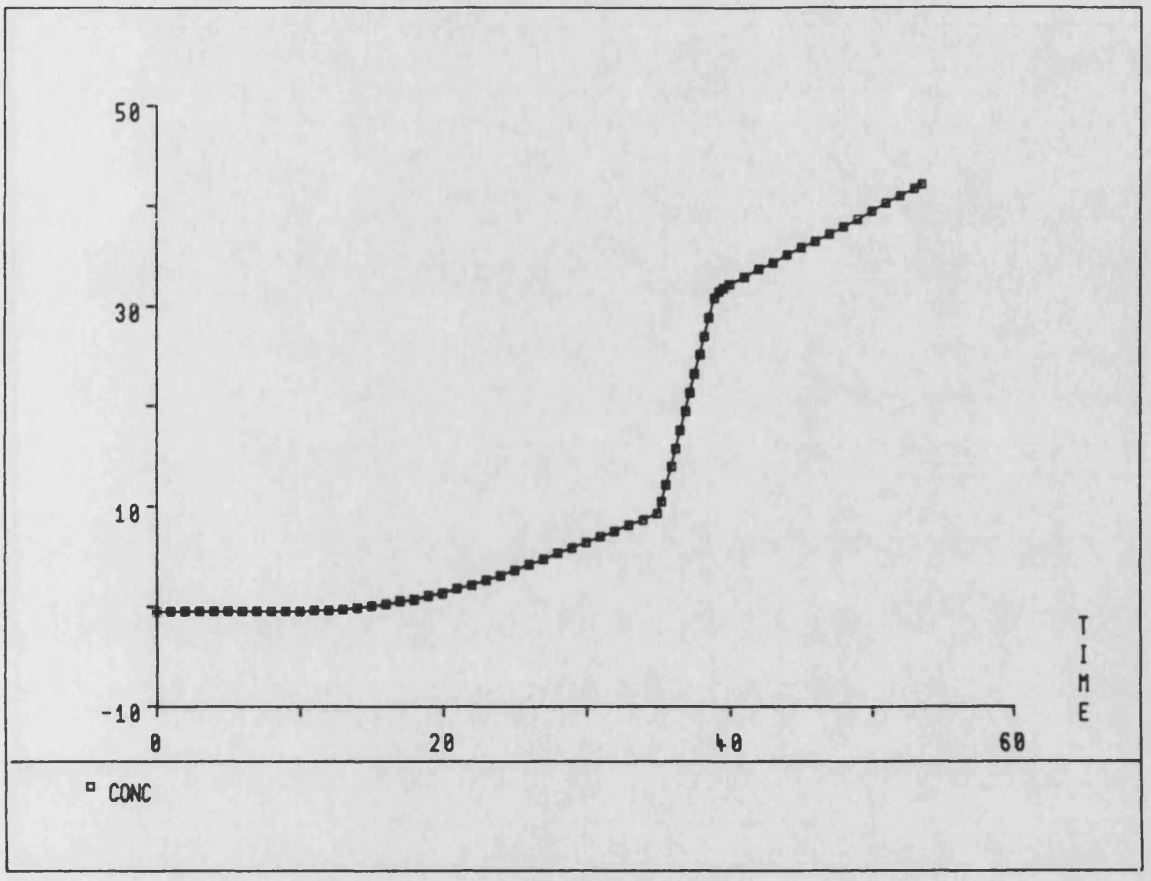
Applied Current of 1.90 mA between 35 and 39 Hours.

1%C PHEMA Film Thickness = 0.105 cm.

Initial Donor Propranolol HCl Concentration = 14.9 mM.

Temperature = 25°C.

Buffer Ionic Strength = 0.039.



Experimental Conditions :

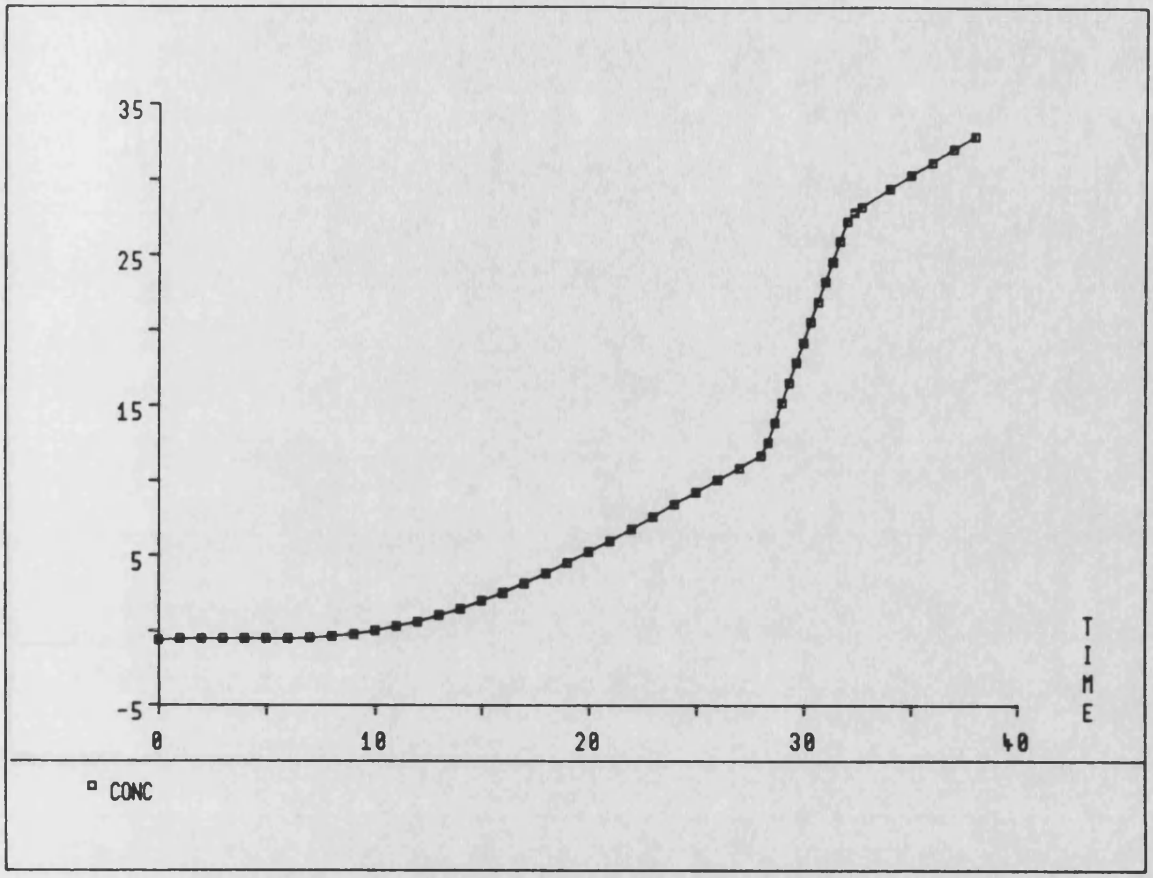
Applied Current of 2.30 mA between 35 and 39 Hours.

1%C PHEMA Film Thickness = 0.107 cm.

Initial Donor Propranolol HCl Concentration = 14.9 mM.

Temperature = 25°C.

Buffer Ionic Strength = 0.039.



Experimental Conditions :

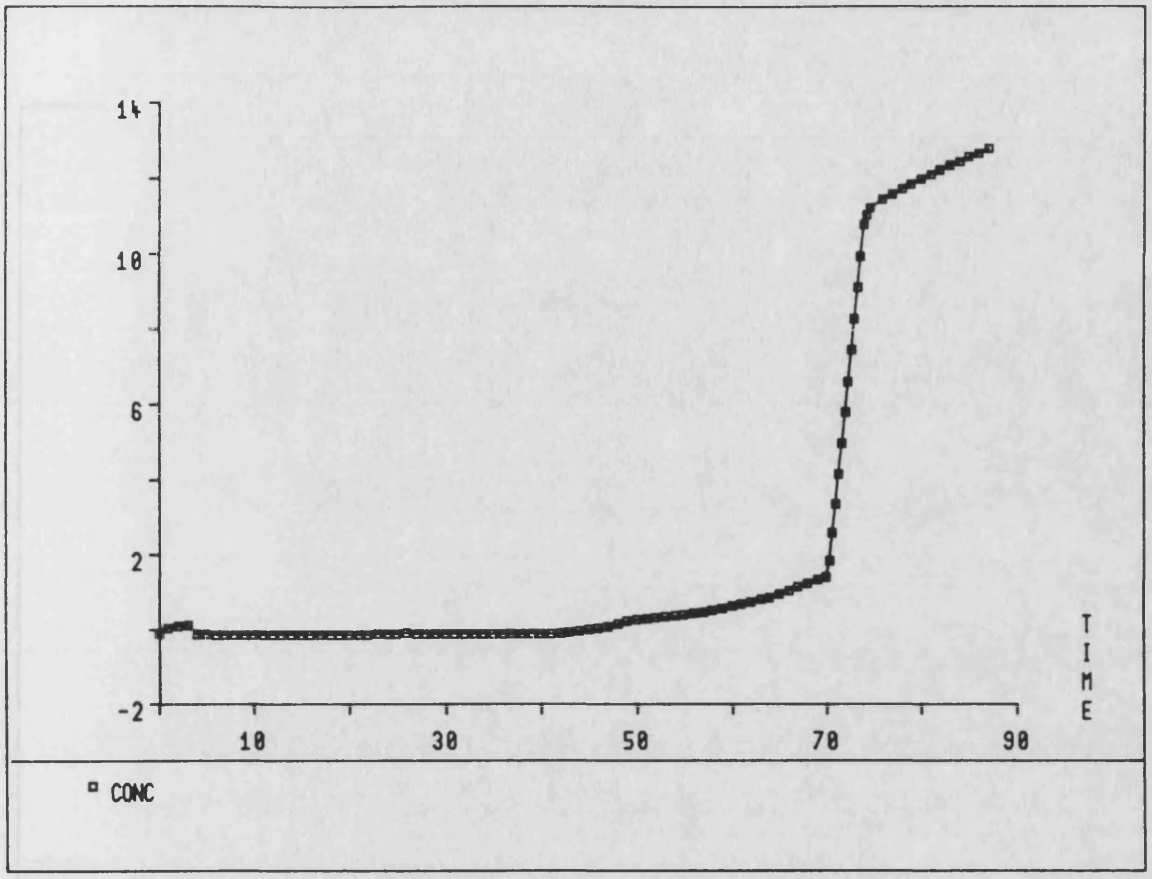
Applied Current of 1.90 mA between 28 and 32 Hours.

0.1%C PHEMA Film Thickness = 0.110 cm.

Initial Donor Propranolol HCl Concentration = 14.7 mM.

Temperature = 25°C.

Buffer Ionic Strength = 0.039.



Experimental Conditions :

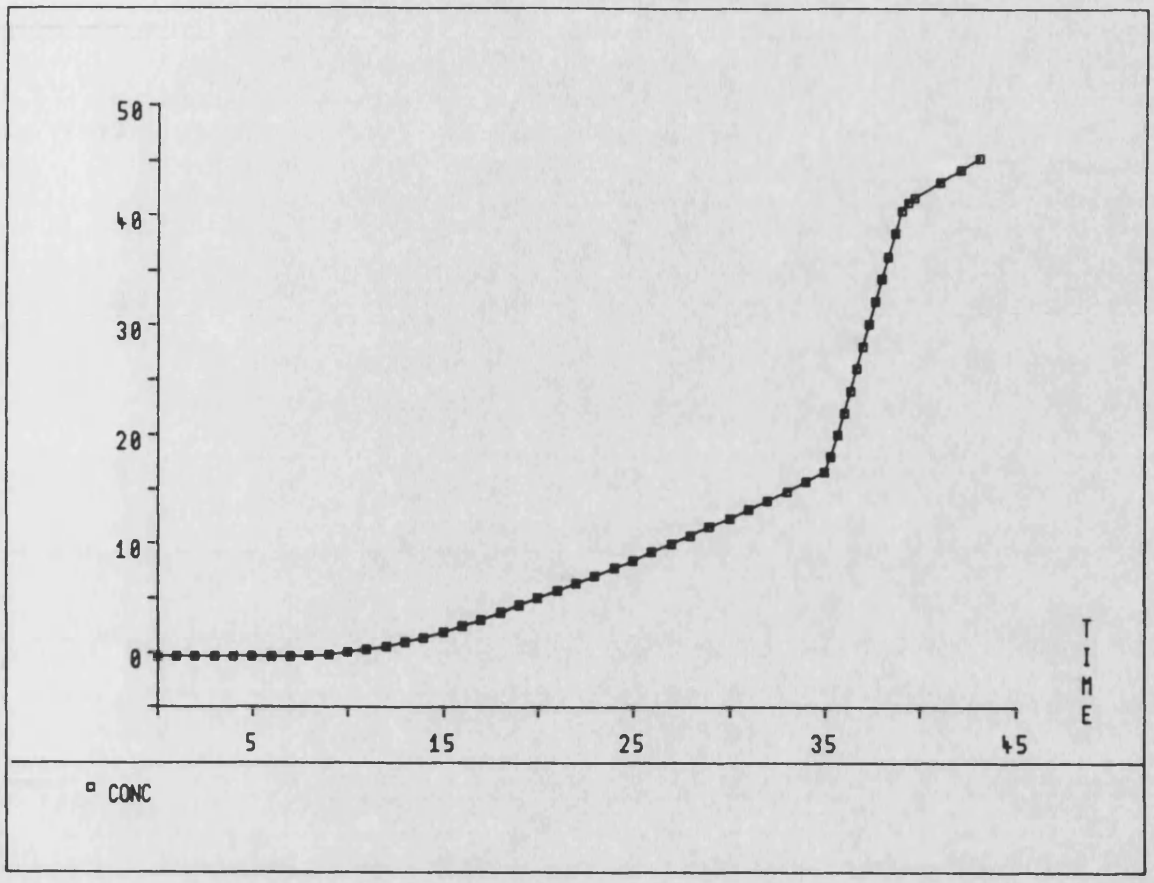
Applied Current of 1.90 mA between 70 and 74 Hours.

7%C PHEMA Film Thickness = 0.092 cm.

Initial Donor Propranolol HCl Concentration = 14.7 mM.

Temperature = 25°C.

Buffer Ionic Strength = 0.039.



Experimental Conditions :

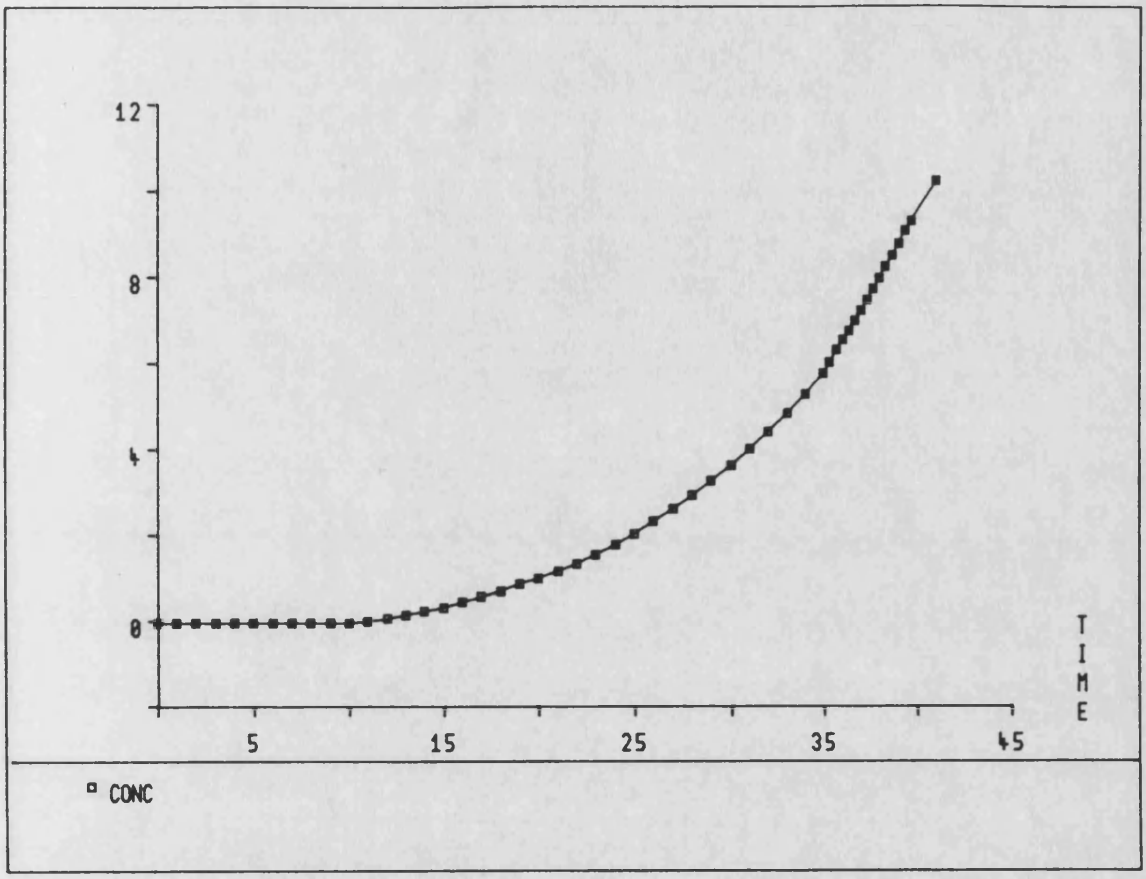
Applied Current of 2.40 mA between 35 and 39 Hours.

1%C PHEMA Film Thickness = 0.089 cm.

Initial Donor Propranolol HCl Concentration = 14.7 mM.

Temperature = 25°C.

Buffer Ionic Strength = 0.039.



Experimental Conditions :

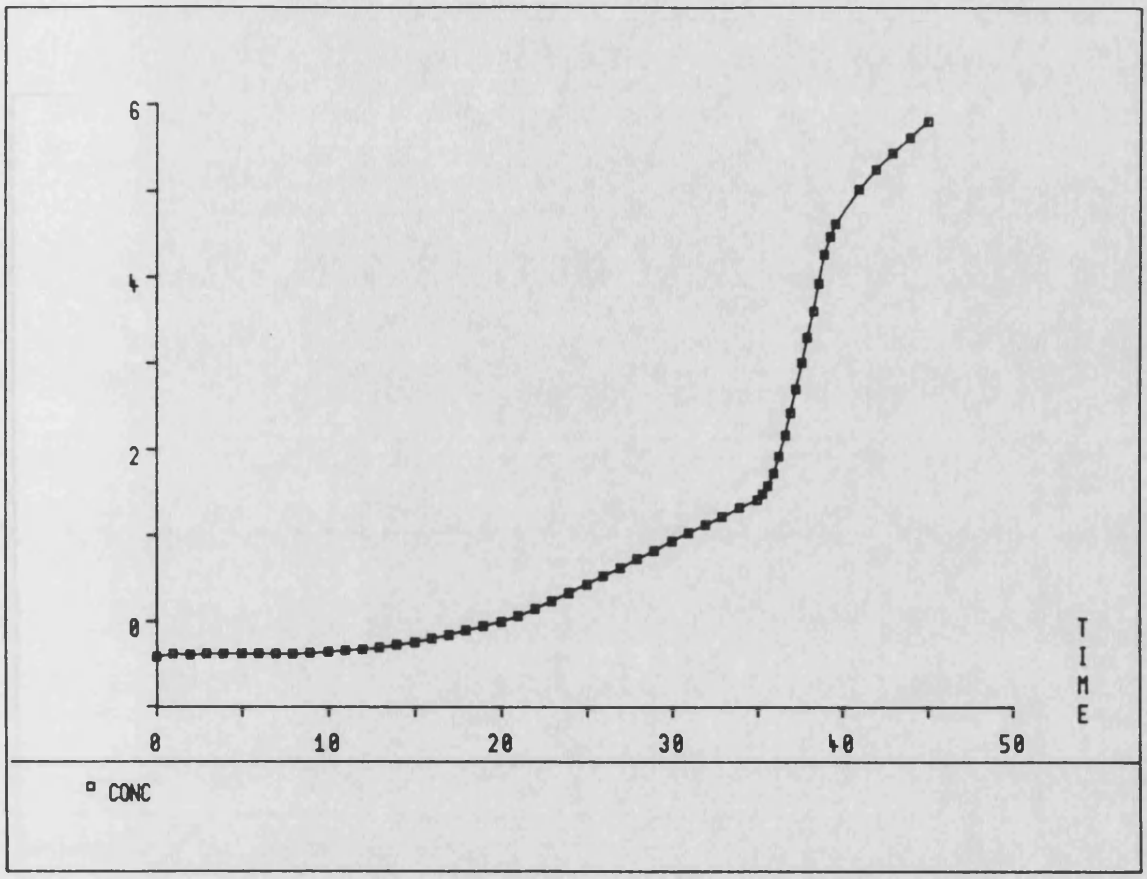
Applied Current of 2.40 mA between 35 and 39 Hours.

1%C PHEMA Film Thickness = 0.104 cm.

Initial Donor Propranolol HCl Concentration = 14.4 mM.

Temperature = 25°C.

Buffer Ionic Strength = 0.200.



Experimental Conditions :

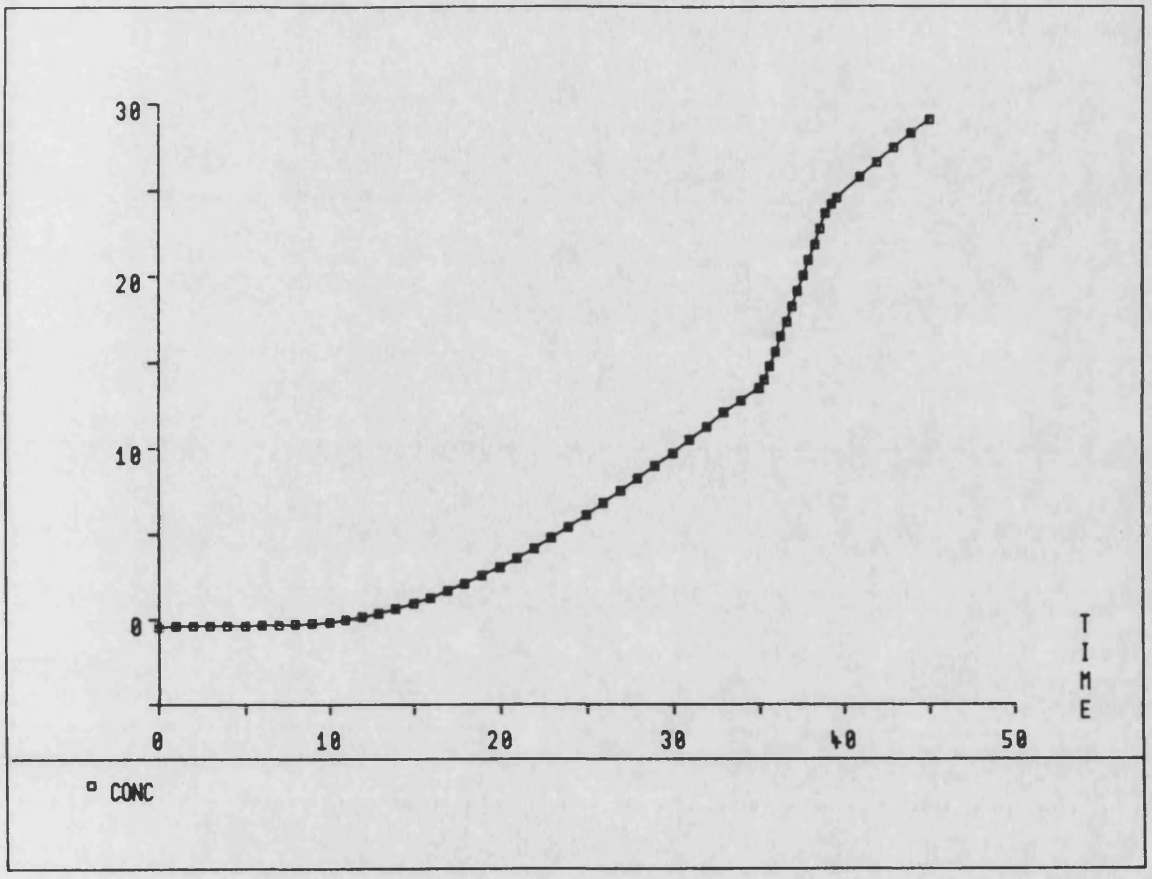
Applied Current of 1.00 mA between 35 and 39 Hours.

1%C PHEMA Film Thickness = 0.097 cm.

Initial Donor Propranolol HCl Concentration = 1.46 mM.

Temperature = 25°C.

Buffer Ionic Strength = 0.039.



Experimental Conditions :

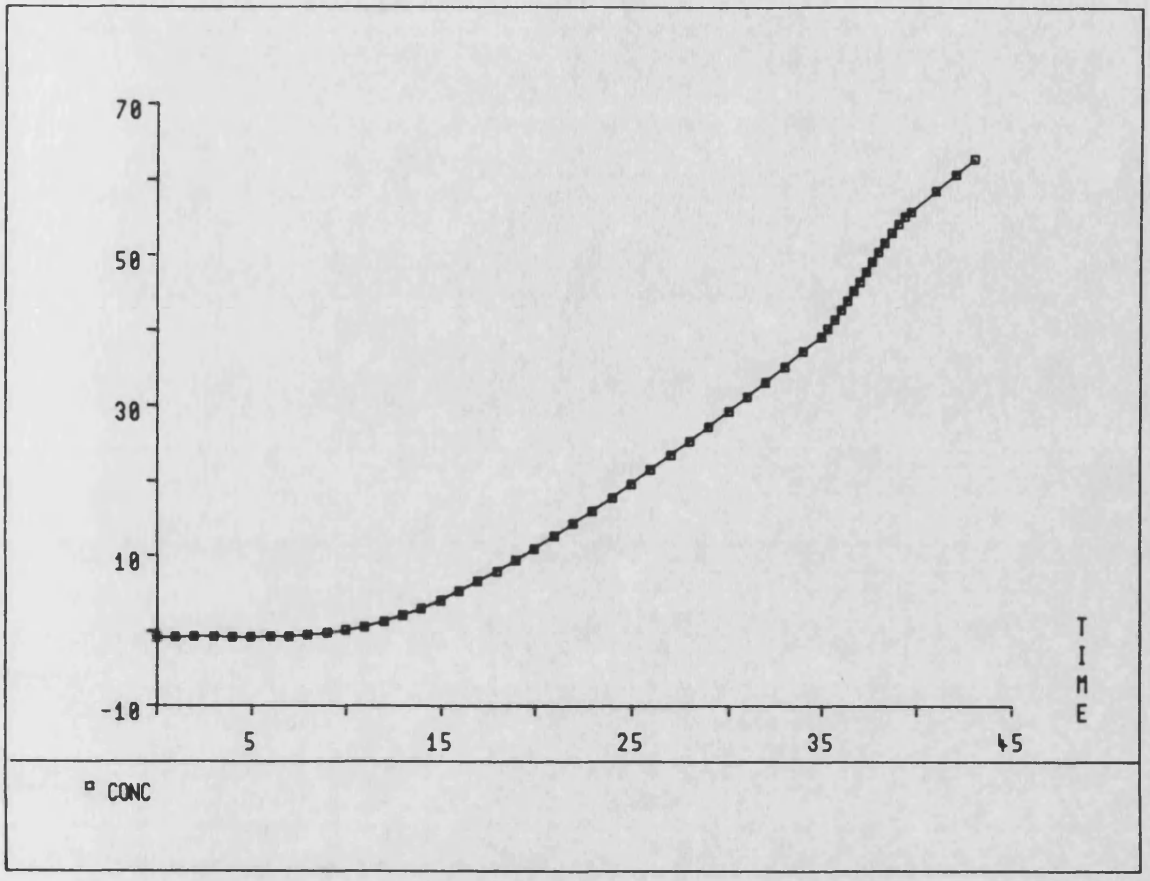
Applied Current of 1.00 mA between 35 and 39 Hours.

1%C PHEMA Film Thickness = 0.096 cm.

Initial Donor Propranolol HCl Concentration = 14.7 mM.

Temperature = 25°C.

Buffer Ionic Strength = 0.039.



Experimental Conditions :

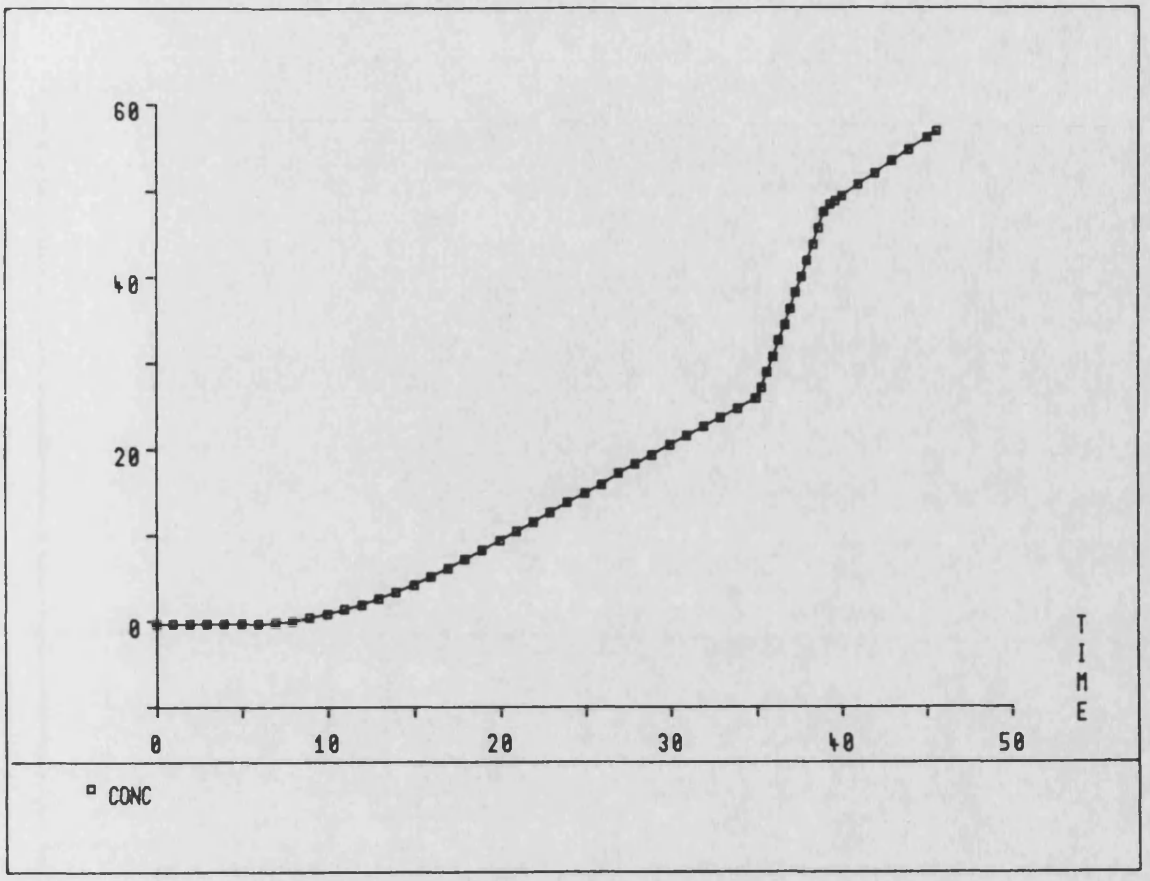
Applied Current of 1.00 mA between 35 and 39 Hours.

1%C PHEMA Film Thickness = 0.097 cm.

Initial Donor Propranolol HCl Concentration = 36.9 mM.

Temperature = 25°C.

Buffer Ionic Strength = 0.039.



Experimental Conditions :

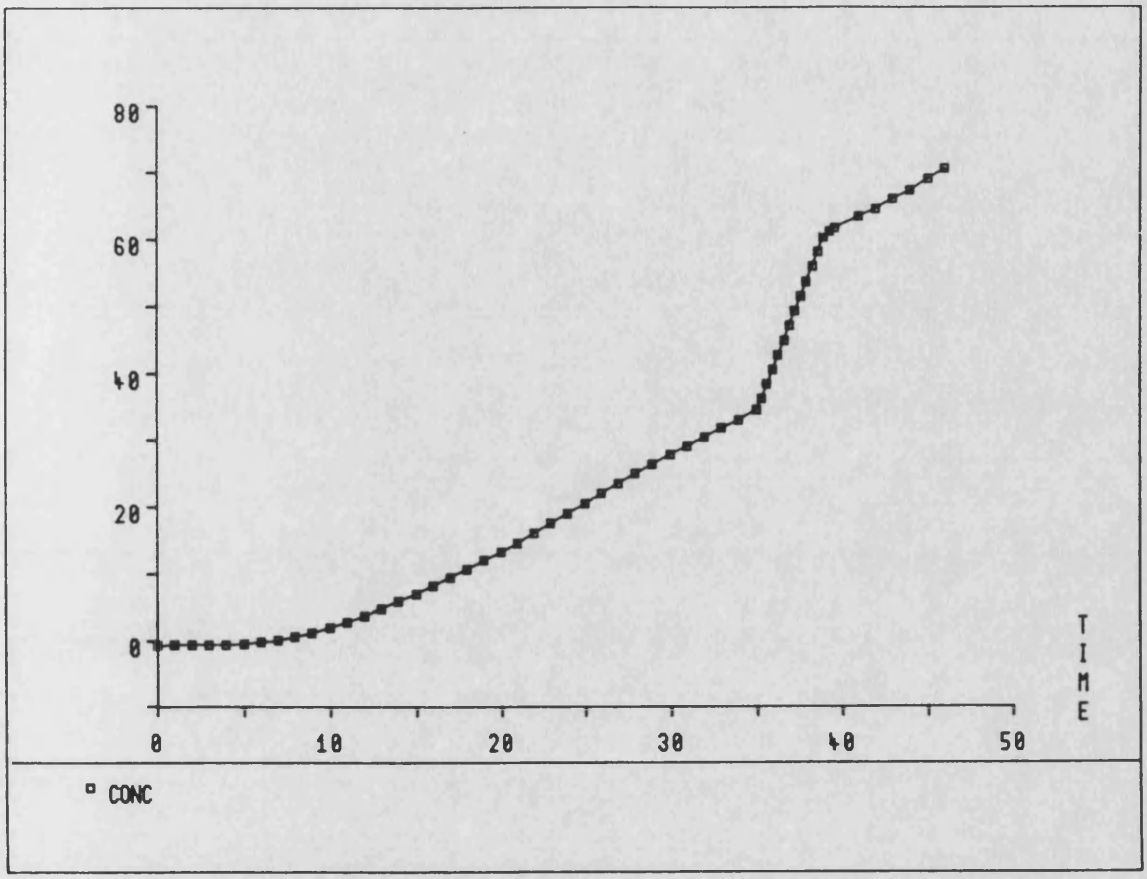
Applied Current of 1.90 mA between 35 and 39 Hours.

1%C PHEMA Film Thickness = 0.090 cm.

Initial Donor Propranolol HCl Concentration = 14.6 mM.

Temperature = 31°C.

Buffer Ionic Strength = 0.039.



Experimental Conditions :

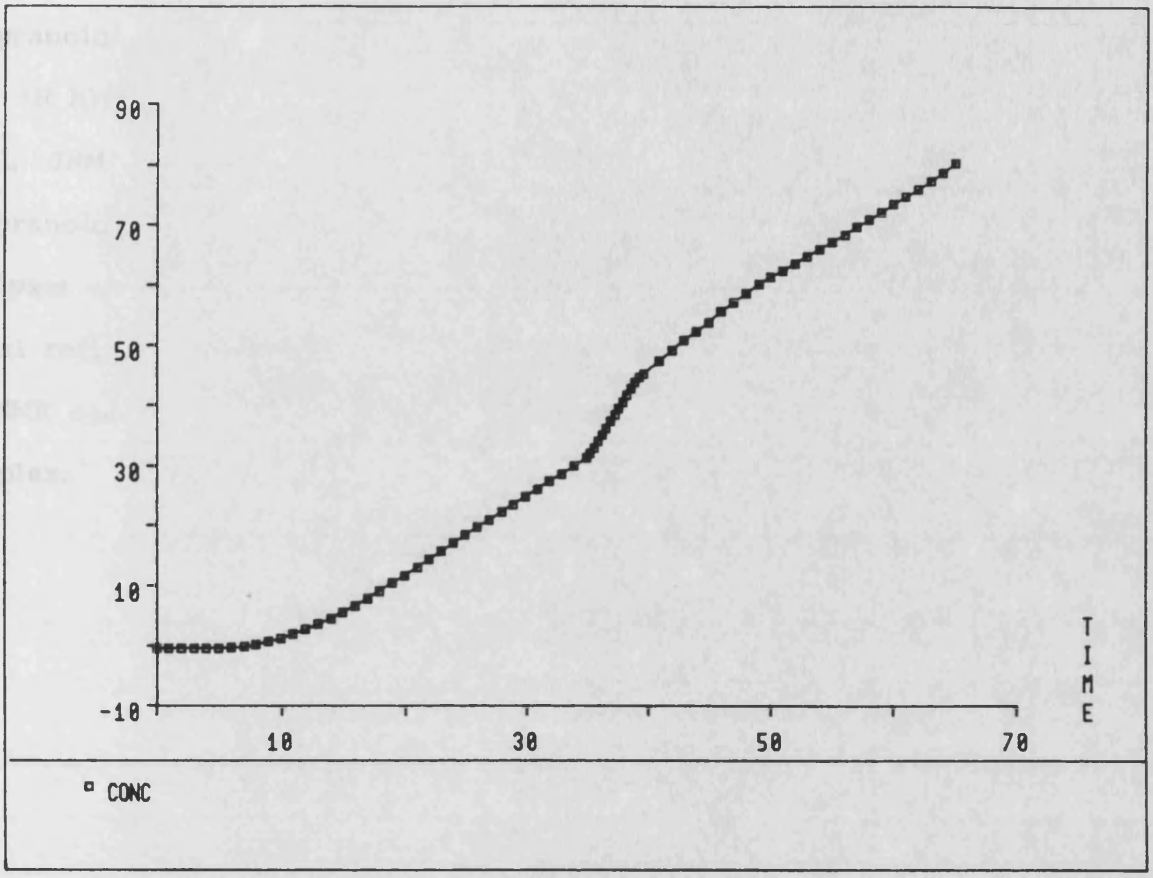
Applied Current of 1.90 mA between 35 and 39 Hours.

1%C PHEMA Film Thickness = 0.094 cm.

Initial Donor Propranolol HCl Concentration = 14.7 mM.

Temperature = 37°C.

Buffer Ionic Strength = 0.039.



Experimental Conditions :

Applied Current of 0.90 mA between 35 and 39 Hours.

1%C PHEMA Film Thickness = 0.099 cm.

Initial Donor Propranolol HCl Concentration = 14.9 mM.

Temperature = 37°C.

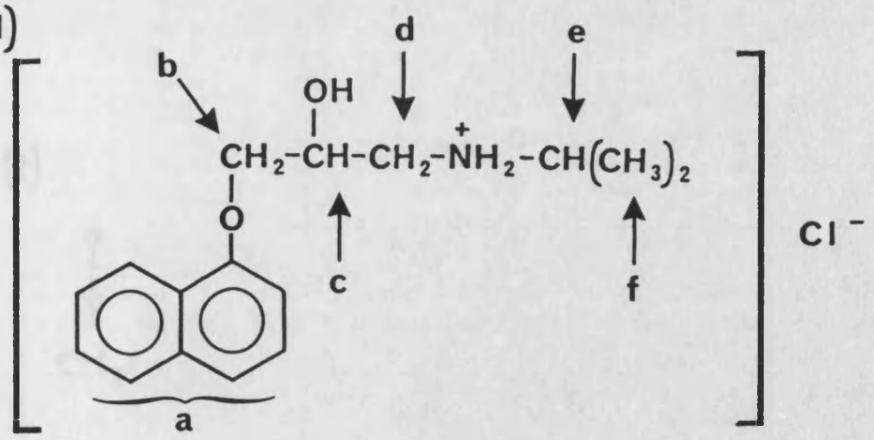
Buffer Ionic Strength = 0.039.

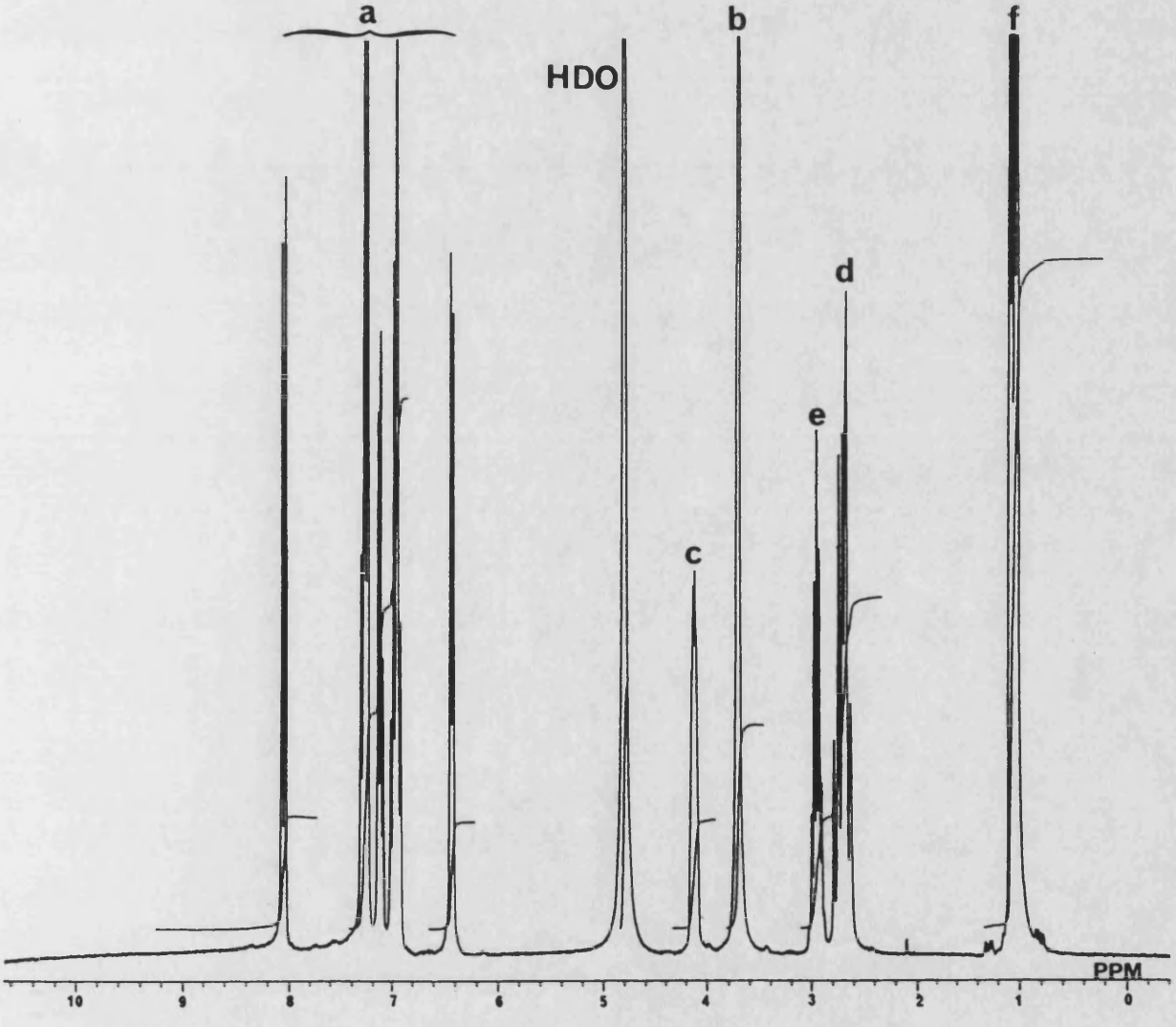
APPENDIX 5

Nuclear Magnetic Resonance Analysis of the Complex between SDDS and Propranolol HCl

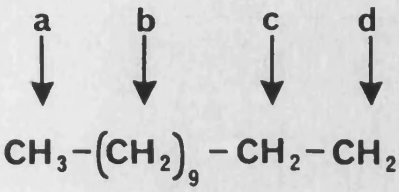
^1H NMR spectroscopy at an operating temperature of 25°C (270MHz, JEOL JNM 270 FT NMR Spectrometer) was used to characterise propranolol HCl (D_2O), SDDS (D_2O), and solid ion-pair ($CDCl_3$). Analysis of the integrals for the solid ion-pair revealed a complex of 1:1 ratio. The following figures show respectively the interpreted ^1H NMR spectra of (1) propranolol HCl, (2) SDDS and (3) ion-pair complex.

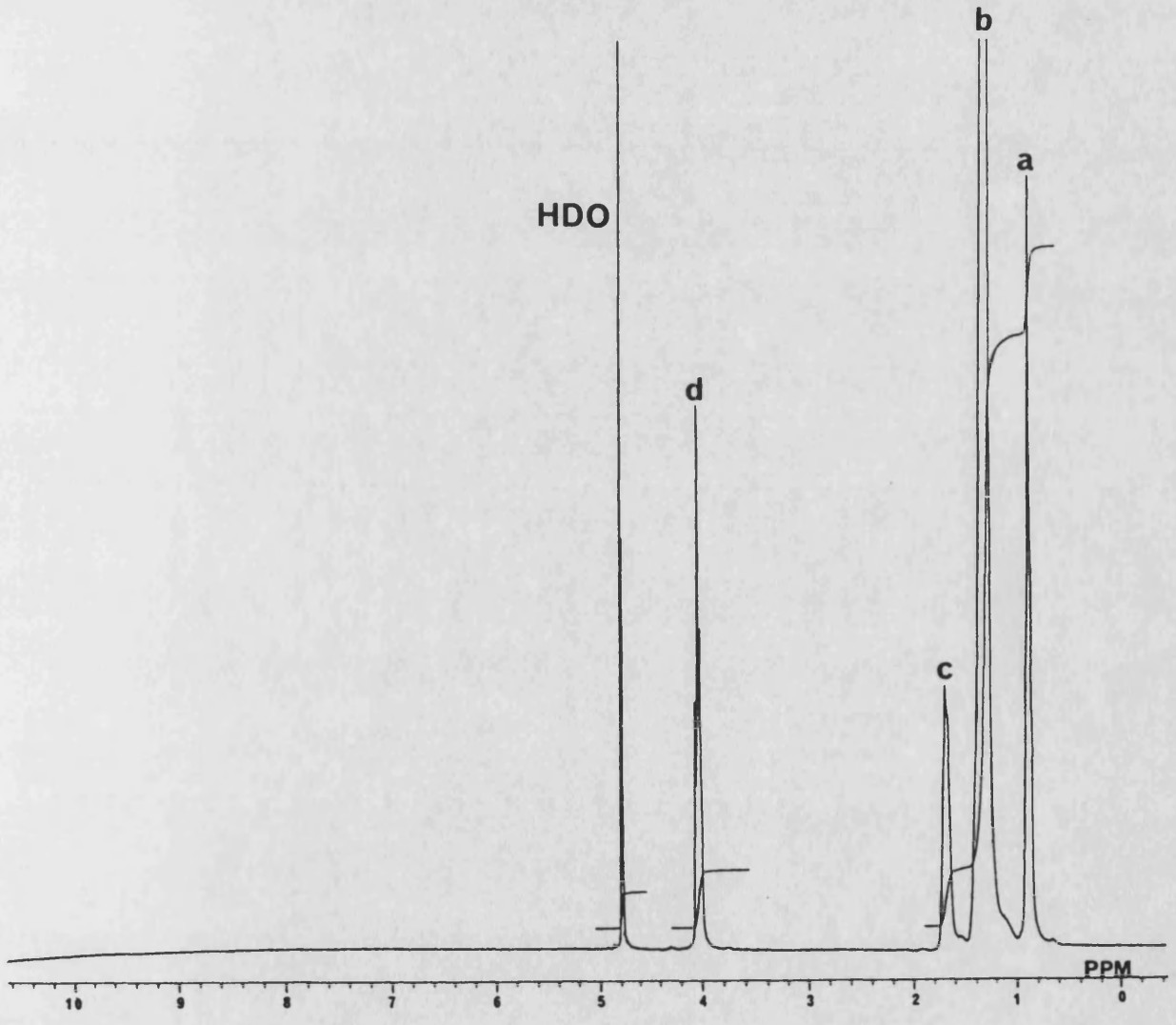
(1)


b
d
e
OH
CH2-CH-CH2-NH2-CH(CH3)2
O
c
f
a
Cl⁻


a
HDO
b
f
d
e
c
PPM
10
9
8
7
6
5
4
3
2
1
0

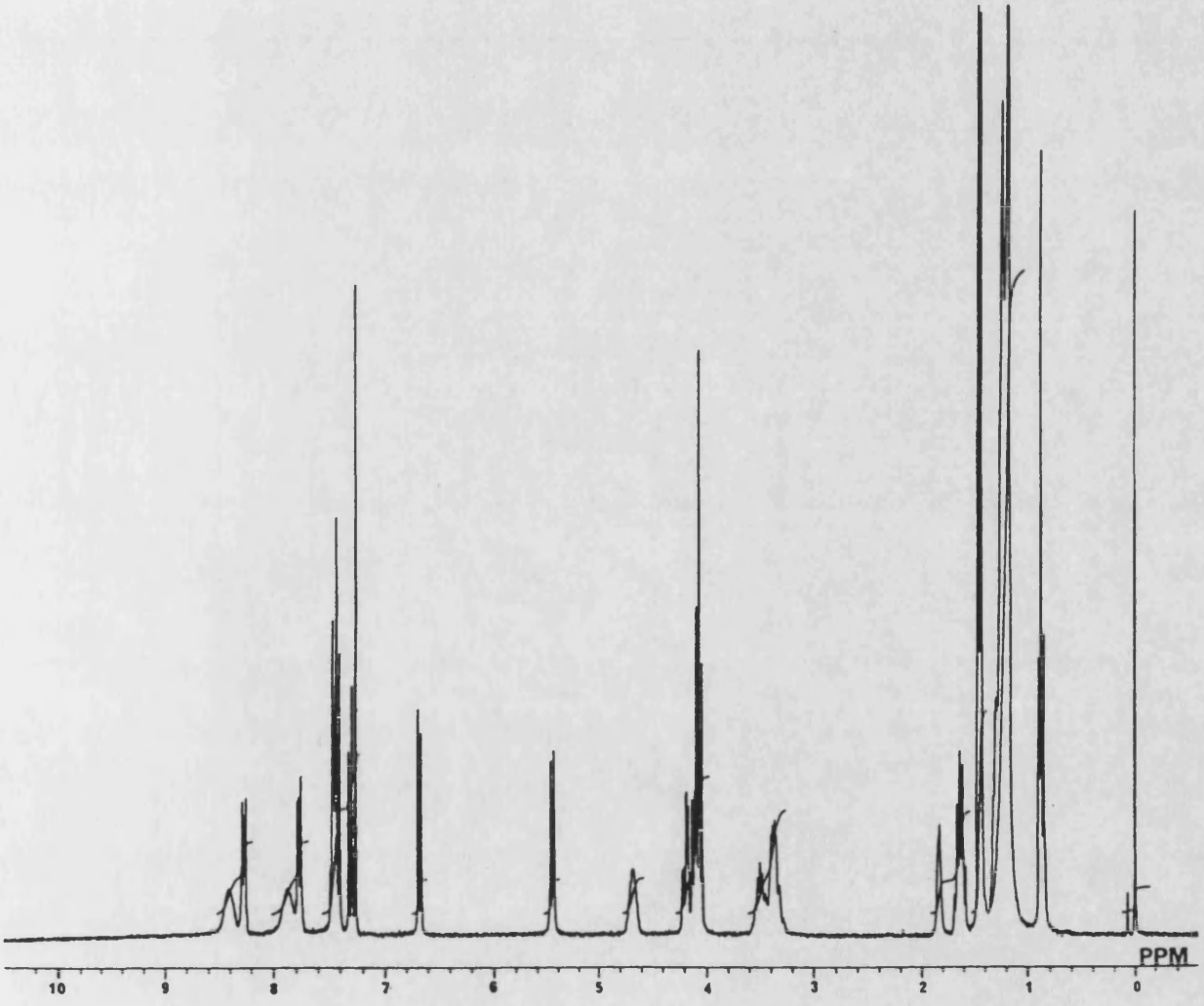
(2)





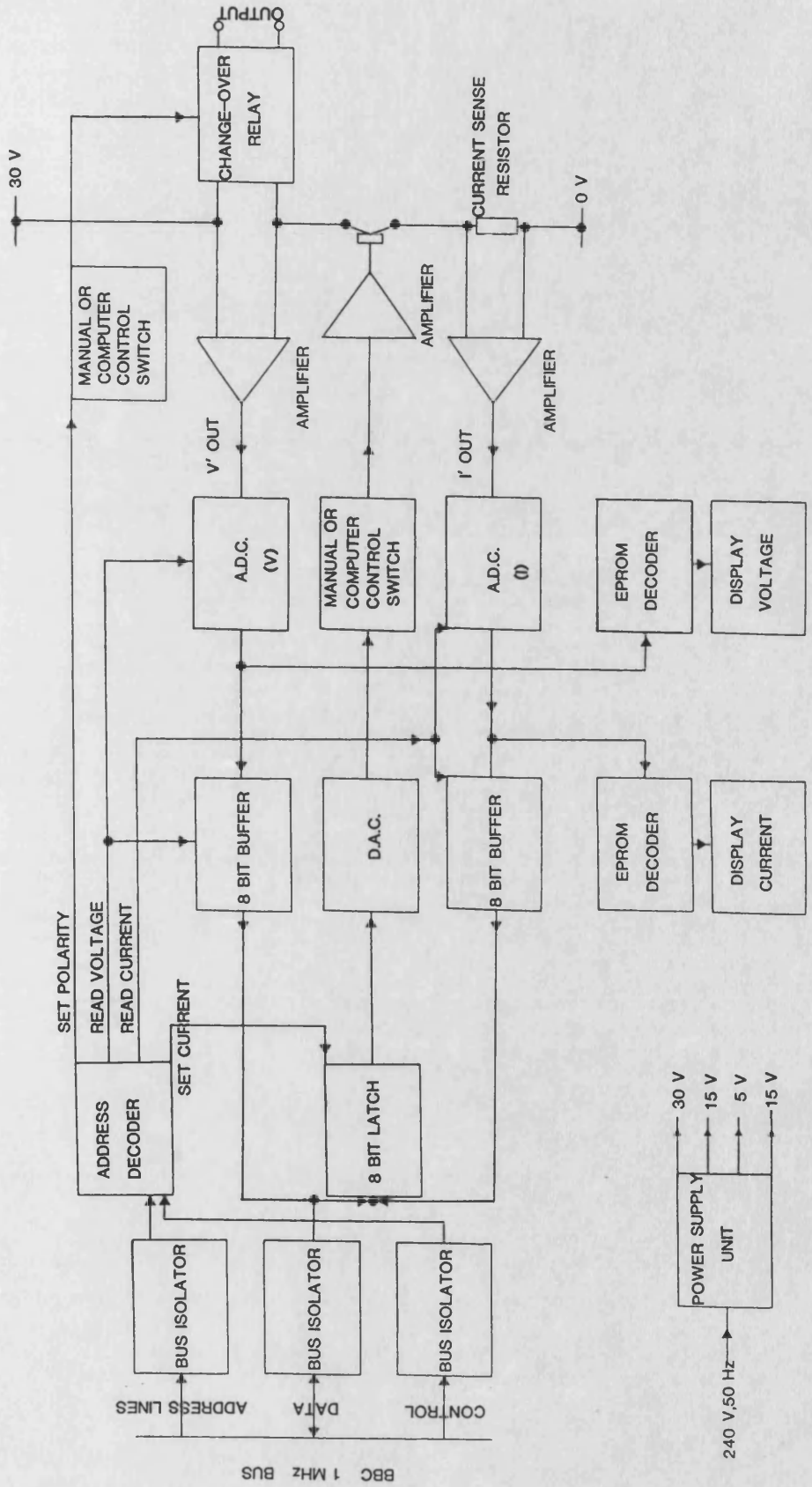
(3)

Ion-Pair



APPENDIX 6

Block Diagram of Constant Current Power Supply



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